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The effect of micro-elevation changes on the distribution of tidal freshwater wetland plant communities

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THE EFFECT OF MICRO-ELEVATION CHANGES ON THE DISTRIBUTION OF TIDAL FRESHWATER WETLAND PLANT COMMUNITIES

An honors paper submitted to the Department of Earth and Environmental Sciences
of the University of Mary Washington
in partial fulfillment of the requirements for Departmental Honors

Thomas Joseph Muratore
May 2017

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Title: THE EFFECT OF MICRO-ELEVATION CHANGES ON THE DISTRIBUTION OF TIDAL FRESHWATER WETLAND PLANT COMMUNITIES

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By
Thomas Joseph Muratore Jr.

Thesis submitted to the faculty of the University of Mary Washington
in partial fulfillment of the requirements for graduation with
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ABSTRACT

Vegetation community structures within tidal freshwater wetlands are subject to control by diurnal tides. Elevation affects the degree of inundation of wetland soils and ultimately controls reduction potentials, a stressor placed on wetland plants. Previous studies have not looked at the affect micro elevation changes have on plant community structures. In order to understand the community structures, relative elevation, reduction potential (Eh), reactive nitrogen, and species diversity were recorded. Sites 1 and 2 were identified on the Pamunkey River and variables were recorded every 2.4 meters and 1.5 meters, respectively. There was a positive correlation between elevation and redox potential and species diversity, while a negative trend was seen between elevation and nitrate concentrations. Relative elevations recorded in this study ranged from -0.08 meters to 0.214 meters. The data suggests that elevation changes over 30 cm significantly affect physiochemical conditions and plant community structure.

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1. Introduction

1.1 Overview

Inland from tidal salt wetlands, but close enough to the oceans to experience tidal influence are the tidal freshwater wetlands. Tidal freshwater wetlands are primarily found along the middle and south Atlantic coasts in the United States of America (Mitsch and Gosselink 2007). These systems are influenced by periodic and predictable tidal inundation that act as stress on organisms found in the wetland reaches. These stresses involve the submergence, saline inputs, and soil anaerobiosis (Mitsch and Gosselink 2007). The same tides also act as a relief for the aquatic system by removing excess salts, reestablishing aerobic conditions, and providing nutrients. The constant fluctuation of tides control sediment dynamics in these tidal freshwater systems and allow for the formation of the wetland levee, a distinguishable feature of these wetlands (Mitsch and Gosselink 2007).

Tides affect the wetland hydrology, which first affects the physical and chemical aspects of the wetlands (Figure 1). This in turn affects the biotic component of the ecosystem, which has a feedback on hydrology. The chemical conditions affected by hydrology are changes to soil chemical properties, such as redox conditions. There are four main impacts that hydrology has on wetland vegetation through changes in physiochemical conditions. First, hydrology leads to unique variations in vegetation composition through the relationship seen in figure 1. Second, hydrology is affected by biota and interactions with primary productivity. Third, hydrology controls organic

matter through the effect it has on primary productivity. Lastly, nutrient cycling is significantly affected by hydrologic conditions and controls the available nutrients for plant use (Mitsch and Gosselink 2007).

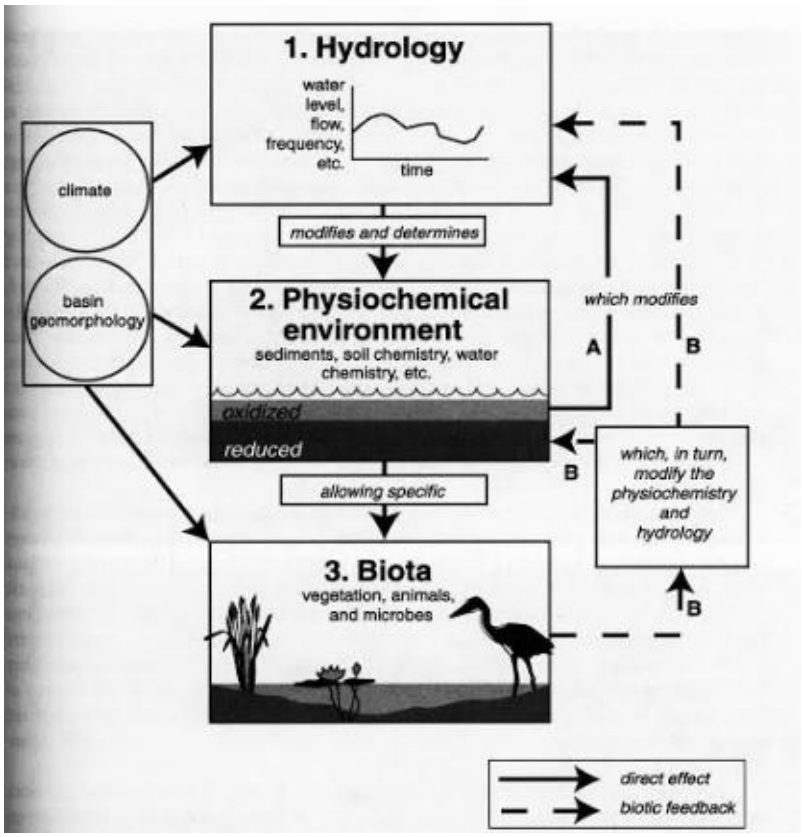


Figure 1: The Effect of Hydrology on Wetland Function. Conceptual diagram illustrating the effects of hydrology on wetland function and the biotic feedback that effects wetland hydrology. (From Mitsch and Gosslink 2007)

The hydrology affecting any part of a wetland is ultimately due to the elevation of that location because elevation controls the duration of inundation. The degree of anaerobiosis is due to the duration of inundation allowing more time for soils to reach highly reduced zones. When soils are inundated with tidal water, anaerobic conditions result. Hydric soils become highly reduced when submerged at a rapid rate. These reducing conditions transform chemical species such as nitrogen and affect the availability of nutrients for plant use. The nitrogen cycle is a key component in plant growth and development and is driven by oxidation and reduction conditions. When oxygen reduction is the dominant metabolic process (respiration), NO_3^- levels are high. As the reduction potential begins to drop into the nitrate reduction zone, NO_3^- levels are quickly depleted, then Fe^{3+} reduction begins. An opposite trend is seen with $NH4^+$: concentrations of ammonium increase as reduction potentials drop because of the accumulation of NH_4^+ due to the lack of oxygen for nitrification (Mitsch and Gosslink 2000).

The result of the combination of hydrology and physiochemical conditions ultimately affect primary productivity and variations in plant community structure due to stress placed on the vegetation (Simpson et al. 1983; Mitsch and Gosselink 2007, 2000; Odum 1988). Hopfenberger (2007) explored the question why annual species exist in tidal freshwater wetlands that were dominated by perennial species. Competition between annuals and perennials may exist, but abiotic factors drove vegetation composition. Annuals were able to withstand abiotic stresses better than perennials

(Pezeshki and DeLaune 2007). However, annual species were shown to be more negatively affected by nutrient inputs than perennials. Abundance of perennials increased with nitrogen fertilization while annuals decreased with nitrogen fertilization (Baldwin 2013).

In particular, the annual species *Aeschynomene virginica* (*A. virginica*; Fabaceae) was studied to understand physiochemical conditions role in its distribution throughout the wetland. *A. virginica* is a rare aquatic plant that may also be impacted by soil reduction and N forms in these wetland soils. *A. virginica* is found in tidal freshwater wetlands of the Mid-Atlantic. It inhabits estuarine meander zones of rivers where sediments transported from upriver settle out and marshes form (United States 1995). *A. virginica* is found in areas that are flooded twice daily and the elevation reaches the upper limits of tidal fluctuation. *A. virginica* establishment decreases as maximum water depth increases (Griffith and Forseth 2003). The distribution of *A. virginica* also relies on seed dispersal for site colonization. It has been shown that A. virginica seeds do in fact disperse from existing populations (Griffith and Forseth 2002) and establish new population sites (Griffith 2014. It is generally found on levees and in areas that are sparsely vegetated due to natural disturbances such as ice scouring, sediment accretion, and muskrat eat outs (United States 1995).

Wetland elevations and plant distribution changes may be particularly important in light of rising sea levels due to global temperature increases. The areas inhabited by these plants are under pressure from sea level changes (United States 1995). As sea level increases wetland elevations will decrease leading to more submerged soils,

removing habitat and increasing chemical stresses. These factors will affect wetland health. The appearance of *A. virginica* could be an indication of good wetland health when present (Griffith 2014).

1.2 Objectives and Approach

This study examines the tidal and soil parameters that may control plant community structure and the occurrence of *Aeschynomene virginica* ((L.) Britton, Sterns & Poggenb. (*A. virginica*, Fabaceae) within tidal freshwater wetlands. The specific objectives of this study are to (1) determine the relative elevation of known *A. virginica* sites, (2) quantify soil O_2 levels throughout tidal cycles to understand soil reduction potentials within and among known *A. virginica* sites, (3) analyze concentrations of inorganic nitrogen in soils at known *A. virginica* sites, and (4) compare physiochemical conditions such as elevation and nitrogen species to distributions of annuals and perennials at known *A. virginica* sites.

1.3 Research Significance

The proposed study will be the first to examine how elevation changes over centimeters affect wetland vegetation structure. Interestingly, rising sea levels on the Atlantic coast have resulted in vegetation shifts in the Chesapeake Bay (Mitsch and Gosslink 2000) and changing parameters such as redox potential and nitrogen speciation over small elevation changes may be the factors that control shifts in vegetation structure. Future increases in sea level may alter vegetation community structure again. A previous study looked at the effect elevation has on nitrogen speciation and

vegetation community structures over elevation changes of m. Nitrate concentrations and annual plants were shown to increase with elevation (Hopfensperger et al. 2009). This study will determine if the changes over elevation ranges of 20 – 30 cm are different on reduction potentials, nitrogen speciation and vegetation community structure.

Findings in this paper may be useful in predicting changes to physiochemical conditions and how vegetation communities respond to rising sea levels. Future changes in vegetation community structure will alter how tidal freshwater wetlands function and the services provided by them.

Changes in vegetation community structure may have particular significance for *A. virginica.* Like other wetland plants, changing physiochemical conditions may affect the distribution of *A. virginica. A. virginica* is said to be threatened by sea level rise as changes in elevation add stress on the already threatened plant. A previous study showed that elevation in the wetland was a controlling factor on *A. virginica.* This greenhouse study looked at the germination and survival of *A. virginica* in wet, waterlogged, and submerged soils and found that germination and survival was most successful in "wet" soils (Griffith and Forseth 2003). But, no study has evaluated the *in situ* elevation ranges the plant inhabits. This study will measure soil water saturation through the availability of O_2 within wetland soils. O_2 availability could be significantly affected by elevation due to its control over hydrology. The hydrology within the wetland will influence the reduction potential at a particular site and the plant communities present.

2. Background

2.1 Tidal Freshwater Wetlands

Tidal freshwater wetlands are a dynamic environment that serves many purposes such as providing a habitat for a large diversity of organisms, many of which are only found in tidal freshwater, and recycling large amounts of nitrogen. These wetlands are subjected to many threats that could alter the distribution of wetland species. Major threats to wetlands are increased nutrient runoff from agricultural lands, habitat loss from development and sea level changes (United States 1995). Increase in nutrient runoff could alter the balance of perennials and annual plants by increasing levels of nutrients such as NO_3^-, which has been shown to control perennial versus. annual distribution (Baldwin 2013). A change in sea level could remove habitats that are specific for species while also altering elevation within the wetlands (Sharpe and Baldwin 2012; Woo and Takekawa 2012). These threats have implications for wetland functions including altering plant biomass and thereby reducing nutrient retention capacities (Engelhardt and Ritchie 2001).

2.2 Balance of Annuals and Perennials

Within tidal freshwater wetlands, high standing biomass of perennials and tidal inundation were identified as two important variables restricting the distribution of annual wetland species (Parker and Leck 1979, 1985; Simpson et al. 1983). Perennial plants are strong competitors due to their ability to maintain roots and uptake nutrients while annual plants rely on seed dispersal and available open habitats (Baldwin 2013).

Because different variables control the distribution of wetland species, some conditions could favor the growth of annuals over perennials.

It was shown that annuals are able to compete for habitat with perennials through abiotic factors that act as a disturbance in the wetland. The abiotic factors allow for various disturbances to alter vegetation, leading to a balance of annuals and perennials in wet wetland. Changes in the abiotic factors within the wetland could alter vegetation community structures and ultimately wetland function (Hopfesnsberger 2007). Abiotic factors such as nutrient inputs have been shown to alter percent covers of annuals versus perennials. Baldwin (2013) showed that perennials increased in plots fertilized with nitrogen while annuals decreased.

2.3 Stress Associated with Tidal Inundation

Within a wetland, tidal inundation times of soils have also been shown to dislodge seedlings of annual plants, making it difficult for establishment (Griffith and Forseth 2003). These inundation times are also important in controlling the distribution of wetland species through seed dispersal. Seed dispersal in flowering plants represents a key process in the regeneration and establishment of plant populations. Seed dispersal patterns play important roles in 1) the number of new individuals added to a population, 2) the spatial arrangement of individuals within a local population, and 3) the potential to establish new populations in suitable habitat patches (Griffith 2002).

Other variables associated with soil inundation times have been shown to affect wetland vegetation composition including the submergence of soils. Excess water in

wetland soils is a major factor that affects plant survival and functioning. These affects include physical, chemical, and biological processes, which put stress on plants (Gabriel and Patrick 1978; Gabriel et al. 1991; Pezeshki and DeLaune 2012). Physical stresses include restriction of atmospheric gas diffusion into the soils, which leads to depletion of soil oxygen and accumulation of carbon dioxide (Jackson and Drew 1984; Greenway et al 2006). Chemical processes that follow this depletion of oxygen are denitrification, reduction of iron, manganese and sulfate and changes in soil pH (Gabriel et al. 1991). These changes in soil chemistry are the stress factors that could affect plant function, survival and occurrence. The stress placed on a plant due to the depletion of oxygen primarily affect plant roots: a decrease in root porosity and a degradation of root systems, which may affect plant nutrient uptake (Pezeshki and DeLaune 2012).

The inundation of wetland soil is the result of diurnal tides. Twice daily, water levels are raised and soils become inundated. The degree to which these soils become inundated is related to the elevation of a specific site in a wetland. The inundation of wetland soils greatly affects the levels of oxygen that is available for respiration. As soils are inundated, air pockets within the soil matrix fill with water, depleting soils of gases. Areas that are higher in elevation will experience less water inundation relative to low elevation areas. Low-lying areas will be the first to be inundated and the last to be exposed (Mitsch and Gosselink 2007; Pezeshki and DeLaune 2012).

2.4 Soil Redox Potential and O_2

Terms such as waterlogged and flooded have previously been used to describe the inundation of these soils (Pezeshki and DeLaune 2012). However, this does not quantify the degree of saturation. In order to describe the degree of saturation within a wetland soil the redox potential can be measured. Reduction potential is a measure of the likelihood of a chemical species to acquire electrons and thereby become reduced. A series of reactions takes place upon soil flooding which leads to low soil redox potential (Eh, mV) conditions (Pezeshki and DeLaune 2012). The depletion of oxygen, because of the loss of gases in soil pore spaces due to replacement by tidal waters and remaining O2 by soil microbial organisms, leads to reduction in soil oxidation-reduction potential (Eh). A series of chemical changes in the soil results. Soils depleted of oxygen have an Eh below 400 mV (Grunwald 2017).

Roots and rhizomes in flooded wetlands obtain oxygen through gas-phase transport from the shoot system, internal photosynthetic production, or atmospheric oxygen through aerenchyma tissue in roots stems and leaves (Kludze et al. 1994; Pezeshki and DeLaune 2012). The effectiveness of the gas transport is dependent on two factors: (1) root length and root porosity and (2) the oxygen demand along the diffusion path (Luxmoore et al. 1972; Pezeshki and DeLaune 2012). These two factors will decrease the amount of oxygen that is available to plant roots in inundated soils. The decrease in oxygen has a negative effect on plant respiratory capacities, which has a direct effect on plant growth and biomass (Pezeshki and DeLaune 2012). The effects of reduction in Eh on plants are more drastic on roots systems then shoots. The low Eh

affects root elongation, which leads to shallow root systems (Pezeshki and DeLaune 2012). It has been reported that root integrity degraded in as little as 10 hours after initiating inundation treatments (Jackson et al. 2003; Pezeshki and DeLaune 2012). This degradation of roots can lead to low levels of nutrient uptake and an increase in toxic compounds in root tissues, which decreases net photosynthesis (Pezeshki and DeLaune 2012).

The correlation between redox potentials and elevation within tidal freshwater marshes are likely due to differences in inundation times. Areas that are higher in elevation will experience less inundation, which means soil would be in an oxygen depleted state for a shorter time. Areas lower in elevation will be in an oxygen depleted state for a longer period of time because the time that the tide inundates soil is longer. As tidal water level recedes, the Eh value will become more positive, indicating a more aerobic environment (Seybold 2002). This could mean that soil in low elevation areas experience lower (more negative) redox potentials compared to higher (less negative) relative elevation areas within the tidal wetland.

Primary electron acceptors change with reduction potentials present in waterlogged soil. Soil redox values range from above 600 mV to below -200 mV, in wetlands. Above 400 mV is said to be oxidized soils where -200 mV is said to be highly reduced. +200 mV is the threshold for nitrate reduction and is described as moderately reduced soil (Grunwald 2017) (Figure 2).

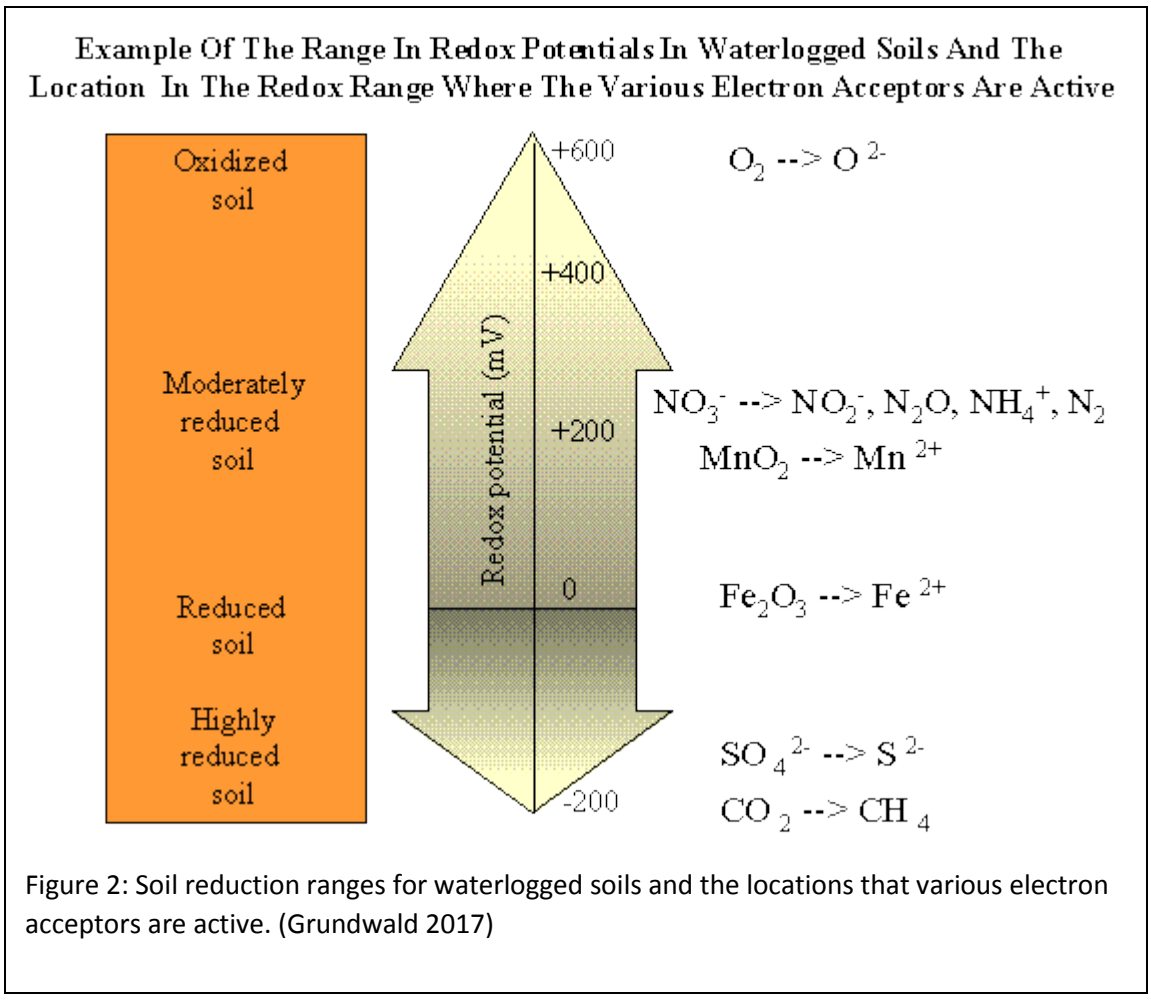


Figure 2: Soil reduction ranges for waterlogged soils and the locations that various electron acceptors are active. (Grundwald 2017)

When reduction potentials are low, respiration is not favored and other metabolic pathways, such as denitrification, take place. These alternate metabolic pathways affect many compounds in wetland soils by changing them to their reduced form (Pezeshki and DeLaune 2012). Denitrification and nitrification are examples of this change to compounds. Nitrogen that is usable by plants, such as NO_3^- is an oxidized form of nitrogen, while reduced forms of nitrogen include N_2 and is unreactive with in plant tissues.

2.5 Available Nitrogen

It has been shown that in wetland soils, higher elevations have higher levels of NO_3^- present while lower elevations have lower levels of NO_3^- (Morse et al. 2004; Hopfensperger et al. 2007. This observation could have two implications for the distribution of perennials versus annual plants in a tidal freshwater system: (1) competition for N chemical species and (2) the ability to function under the stress of low reduction potentials. Competition for useable N in a soil is greatly increased as soil redox potential is decreased. The decrease in soil redox potential increases denitrification rates, which reduces oxidized forms of N, such as NO_3^- to reduced N or N_2. Denitrification leaves little NO_3^- to be used for plant function. The competition and relative biomass of annuals and perennials has been shown to be affected by NO_3^- concentrations (Baldwin 2013; Hopfensperger et al. 2009). In Baldwin (2013), it was shown that perennials' percent cover increased with increased NO_3^- levels while annual's percent cover decreased with increased NO_3^-. This could control the distribution of plants throughout wetlands.

Ultimately, the various stresses associated with elevation changes in the wetland control vegetation community structure. Large elevation changes (e.g. meters) have affected perennial / annuals ratios (Hopfensperger et al. 2009). Increased levels of annuals were found in higher elevations while more perennials were found in lower elevations. But, the effect that elevation has on these stresses over smaller elevation changes (e.g. cm) is not well understood. In the coming decades sea level rise will increase one to two centimeters per year (Poore et al. 2011). Over the years these micro elevation changes could alter plant community structure. Understanding how sea level rise will change the stresses places on wetland plant communities will be important in preserving the future of this critical ecosystem and the species that inhabit them.

3. Materials and Methods

3.1 Sampling Design

The study site is located at the Vandell Preserve at Cumberland Marsh (Cumberland Marsh) in New Kent County, Virginia, along Holts Creek (37°33'12.55"N, 76°58'43.35"W)(Figure 3). The Cumberland Marsh is a tidal wetland that contains populations of *A. virginica*. This marsh is owned by the Nature Conservancy and is a protected wetland. *A. virginica* populations on Cumberland Marsh have been closely monitored since 1996 (SJV Monitoring; Griffith personal communication).

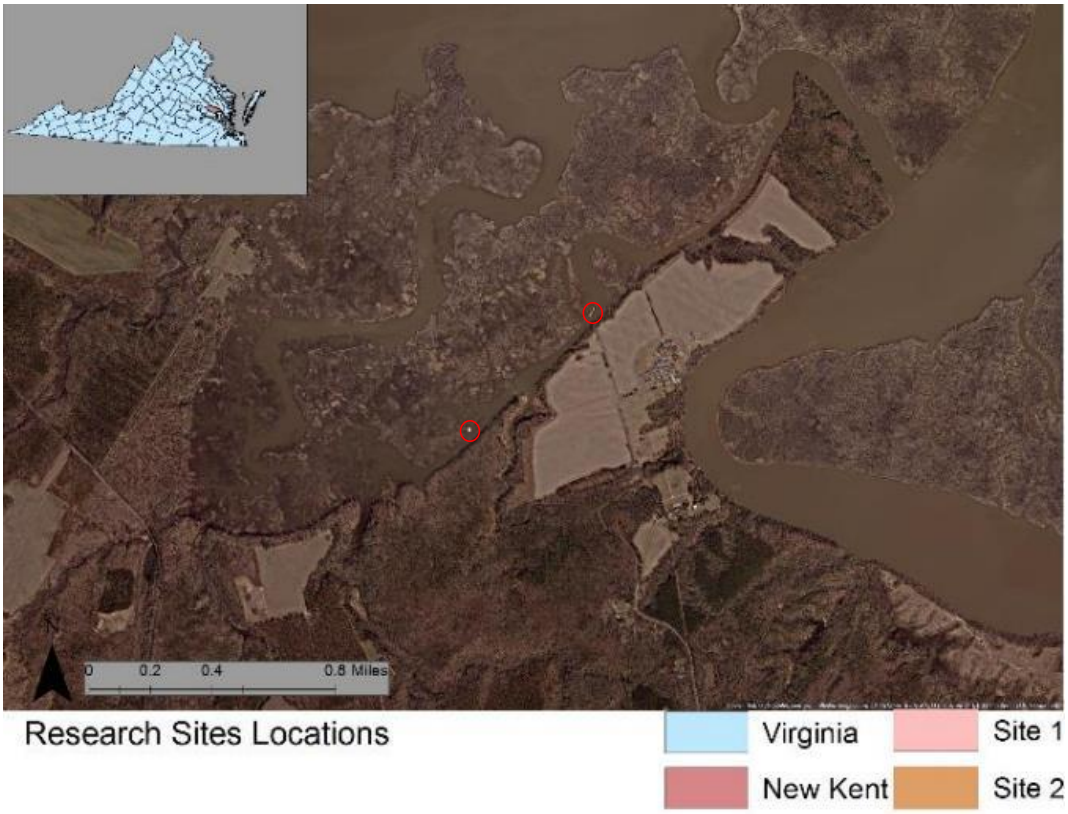


Figure 3: Map of Research Sites in New Kent County. The Vandell preserve at the Cumberland Marsh in New Kent County. Sites 1 and 2 shown on the map for reference.

A. Site 1

B. Site 2

Figure 4: Sites 1 and 2 Transects. A. is a figure of Site 1 with three transects (0101xx, 0102xx, 0103xx). B. is a figure of Site 2 with tree transects (0201xx, 0202xx, 0203xx). Both sites were identified on the Cumberland Marsh in New Kent, Virginia.

Two sites at the Cumberland Marsh were identified from long term *A. virginica* monitoring data from the preserve (SJV Monitoring; Griffith personal communication). Site 1 was located further upstream relative to site 2 (Figure 3 and Figure 4). Each site was systematically sampled with line transects. At site 1, three transects were created 15 m apart which extended 40 ft. parallel to one another into the back marsh. Samples for each parameter were recorded every 8 ft. starting at 0 ft. for a total of 6 samples per transect. At site 2, three transects were created that extended 25 ft. parallel to one another into the back marsh. Samples for each parameter were sampled every 5 ft. starting at 0 ft. for a total of 6 samples.

Sampling sites were given a six number identification, which was used to easily identify each sample location. The first two digits refer to the site location, ie. 01xxxx or 02xxxx. The third and fourth digits refer to the transect number at the site, ie. xx01xx, xx02xx, or xx03xx. The fifth and sixth numbers refer to the sampling location along the previously defined transects and site, ie. xxxx01-xxxx06. All sites are shown in figures 3 and 4. Each location will be sampled for percent cover of annuals and perennials, elevation, soil redox potentials, and NO_3^- levels.

A. virginica plants do not emerge from the vegetation canopy until late August-November and produce very delicate seeds which are easily disturbed (Griffith and Forseth 2003). In order to minimize interference with the germination and growth of *A. virginica* plants during the first portion of its growing season, a system of temporary wooden walkways were constructed. These wooden walkways were moved into and out of the wetland, in order to avoid repetitive disturbances to the soil and plants.

3.2 Relative Elevation

Relative elevation of the wetland was measured by recording a series of water depths every 5 minutes over at least 1 tidal cycle and as many as 8 cycles.. HOBO U20 water level loggers (Onset Computer Corporation, Pocasset, MA) were placed at each location at soil surface and set to log water pressure every 5 minutes. A reference site was created in order to standardize all tidal cycles and water depths throughout the study. The reference site consisted of 2 HOBO loggers located at location 010101 left there throughout the study. One HOBO at this location was affixed above normal tide levels in order to monitor air pressure. A second HOBO was placed at the soil surface directly below the HOBO that was measuring air pressure. The reference site data was collected every day that relative elevations were being measured in the field in order to make comparison to all other transect locations. This site was used as the relative elevation of 0 m.

HOBOware Pro software (Onset Corp. 2016) subtracts the air pressure from the water pressure that gives the pressure of the water pressure depth. Water pressure depth is then converted to water depth within the program. This calculation was done for every sampling location in order to determine the water depth. Water depths were converted into relative m above reference location by subtracting the water depth of the reference location from each of the sampling locations.

3.3 NO_3^- and NH_4^+ Extraction

The most direct measures of the nitrate and ammonium levels within a wetland are through analytical soil nitrate and ammonium extraction. These values will show how much available nitrogen is present at each site location. In order to measure these values, soil samples were taken from the first 6 inches of soil at each site location. Soil samples were stored in clean zip-lock bags and immediately placed on ice in the field. Upon return to the lab, samples were stored at approximately -10°C. Sampling for each site was done on the same day to ensure consistency across nitrogen levels.

Soil NO_3^- and NH_4^+ extractions were based on the protocol laid out by R.L Mulvaney 1996 (Sparks 1996). To begin the extraction process, soils were partially defrosted over two days at approximately 2°C. Once defrosted, soils were moved into clean aluminum pans. Aluminum pans were placed into an oven at 70^C overnight. Dried soil was homogenized using US Standard Testing Sieves to break up aggregates and remove large pieces of organic matter. This ensured that the soil had uniform characteristics for the extraction. 10.0 grams of dried soils were placed into wide mouth Nalgene bottles along with 100.0 mL of KCl. The KCl and soil mixture was shaken overnight. The extraction was stopped the following day by separating the KCl extractant from the soil. Vacuum filtration was performed using a Buchner funnel and sterile filter paper (Fisher Scientific 125 cm) Extractant was filtered off and stored in clean glass jars, which were stored at approximately 2^C. Soil extracts were sent to Virginia Commonwealth University Environmental Analysis Lab. Skalar San++ CFA was used by the lab in order to quantify concentrations of nitrate and ammonium.

3.4 Soil redox potential

A variety of parameters can be used to measure O_2 levels in soil, however continuous measuring and monitoring of these levels can be difficult (Farrell et al. 1991). The soil redox potential is one parameter that measures relative O_2 availability and can be measured with relative ease (Farrell et al. 1991). Soil redox potential gives an accurate measure of the oxygen available in saturated soils and redox electrodes can be constructed in order to measure these values (Farrell et al. 1991; Owens et al. 2005). In order to measure these potentials, full system platinum redox probes with an Ag/AgCl reference probe were constructed.

Platinum probe construction was based on the method in Owens et al. 2005. In order to construct the platinum electrode, 18 gauge platinum wire was cut into segments approximately 6 cm long and cleaned in a 1:1 solution of nitric and hydrochloric acid for 4 hours. Wire segments were stored in deionized water overnight. The following day, Pt wires were soldered to 12 gauge copper wire and the join was completely sealed with a waterproof epoxy (3M Adhesive Sealant 730 UV) leaving approximately 1 cm of Pt wire exposed at the tip. Wires were allowed to dry overnight and seals were tested by checking connectivity of circuits through water.

Ag / AgCl reference electrodes were designed in order to minimize glass parts that could potentially break through repeated used in the field. The protocol for constructing and testing the reference electrodes utilized the methods laid out by Farrell et al 1991. The body of the cell was built using a 60 ml plastic syringe with the

internal reference element being an Ag/AgCl electrode. A 1.25 cm piece of silver wire was soldered to 12 gauge copper wire and sealed in epoxy. The surface of the silver wire was electrolyzed against a Pt probe in 0.1M HCl solution in order to coat the Ag wire surface in Cl. Number 6 sized stoppers were drilled with holes to allow the Ag/AgCl wire to pass through along with 2 glass tubes. One glass tube was designed to allow refilling of KCL solution. The second tube was bent into a U-shape to allow airflow to the internal structure of the cell while keeping water out while submerged. The rubber stopper was placed onto the syringe and sealed with a strong silicon caulk (3M Adhesive Sealant 730 UV). Once the internal structure of the reference electrode was constructed, each electrode was filled with 3.5M AgCl saturated KCl salt bridge solution. Contact between the salt bridge, AgCl wire, and soil matrix was made possible through the addition of a ceramic microtensionmeter, which was attached to the end of the syringe (Farrel et al. 1991).

Each electrode system was tested in a standard Zobell's solution. This solution has a known redox potential of 284 mV mV. The reading of each electrode system when placed in the standard solution was used to calculate the offset reading of each system. Each system offset was the known standard redox potential minus the electrode system reading. This offset was used to standardize each redox potential reading in the field.

Six electrodes were constructed and deployed into the field at a single time. Each transect was sampled and all locations on a transect were sampled during the same tidal cycles. The CR10X was programmed (PC200W software, Campbell Scientific, Logan UT) to allow for the recording of redox potentials every 5 minutes across tidal cycles.

Reference probes and Pt redox electrodes were connected to the datalogger by using 25 feet of copper wire. For standardized purposes, the Pt probe was connected to the high output on the datalogger. In situ, full systems were placed in the soil about 4-6 inches at each of the sampling locations. Data was downloaded in situ (PC200W software, Campbell Scientific, Logan UT) and was later standardized using previously calculated offsets.

3.4.1 Redox Means

Because redox potentials are collected every 5 minutes, central tendency measures can be calculated several ways. Mean Eh was calculated as the mean of all Eh values measured. The mean maximum Eh (mean max Eh) was calculate as the mean of each maximum Eh value across several tidal cycles. The mean minimum Eh (mean min Eh) was calculated as the mean of each minimum Eh value across several tidal cycles.

3.4.2 Redox Ranges

Redox range categories were defined based on the primary electron acceptor in the metabolic pathway and were used in order to assign categories for the degree of reduction in the soil (citation?). These categories were then used to determine the percent time locations spent in each reduction category. Below -100 mV was said to be highly reduced, between -100 and 100 was said to be high denitrification and between

Table 1: Categories of Soil Reduction Ranges Used in this Study. Will be used to determine the percent of time spent in each reduction category.

Reduction Category	Redox Ranges (mV)
Highly Reduced	X<-100 mV
High Denitrification	-100 mV< X <100 mV
Moderate Denitrification	100 mV< X <350 mV
Aerobic threshold	X >350 mV

100 mV and 350 mV was said to be in a moderate denitrification state. Redox recordings higher than 350 mV were above the aerobic threshold (Table 1).

3.5 Species Diversity Measures

Plant abundances were sampled using a square meter plot at each location on each transect. At each location, plants were identified to species and percent cover was estimated for each species. At each location, caution was taken to look for *A. virginica*.

Simpson biodiversity index is the measure of species diversity in an ecosystem. D represents the Simpson- Wiener index and is the measure combining species richness and evenness. (n) represents the percent cover for a particular species in a plot. N represents the percent cover of all species in a plot. The equation below represents the Simpson Biodiversity index (Simpson 1949).

$$D = \frac{\Sigma\ n(n-1)}{\Sigma\ N(N-1)}$$

A Simpson-Wiener reciprocal index was calculated by 1/D. This inverse calculation of the Simpson Biodiversity Index provides easy interpretation of measures. Values are ≥ 1. 1/D = 1 is a plot with just one species while 1/D > 1 is a plot with more than one species and /or more evenly abundant species.

Plants were classified as annuals or perennials using NRCS plant data base (2017) Ratios of annuals to perennials were calculated for each location by dividing number of annuals at each location by the number of perennials.

3.6 Statistical Analysis

Statistical analyses were done using IBM SPSS Statistics 24 (IBM Corp. Armonk NY) to determine trends between elevation, soil chemical parameters, and plant community richness. Pearson correlation was used to determine significance between variables. P-values ≥ 0.05 were considered significant.

4. Results

4.1 Site comparisons for All Measures

4.1.1 Relative Elevation

Elevations of wetland sites were relative to location 010101, which was set at 0.0 m. The mean elevation of site 1 was 0.12 ± 0.029 m. The mean elevation for site 2 was 0.06 ± 0.018 m above location 010101 (Table 2). The minimum elevation for transect 0101xx was 0 m found at 010101 and maximum elevation was 0.21 m and found at 010106. The minimum elevation for 0102xx was 0.03 m found at 010201 and the maximum elevation 0.15 m was found at location 010206. The minimum elevation for transect 0103xx was -0.03 found at 010301 and the maximum elevation 0.15 was found at location 010304. (Figure 5; Table 2) The mean elevation for transects 0101xx, 0102xx, and 0103xx are 0.143 m, 0.102 m, and 0.115 m, respectively. Figure 5 shows elevation profiles of site 1. The minimum elevation for transect 0201xx was -0.002 m found at location 020106 and the maximum value 0.14 m was found at location 020103. The minimum elevation for transect 0202xx was -0.02 m found at location 020205 and the maximum value was 0.1 m found at locations 020202 and 020203. The minimum

elevation for transect 0203xx was -0.08 m found at location 020301 and the maximum value was 0.15 m found at locations 020303 and 020304 (Figure 6; Table 3). The mean elevation for transects 0201xx, 0202xx, and 0203xx were 0.045 m, 0.068 m, and 0.072 m, respectively. Figure 6 shows elevation profiles of site 2.

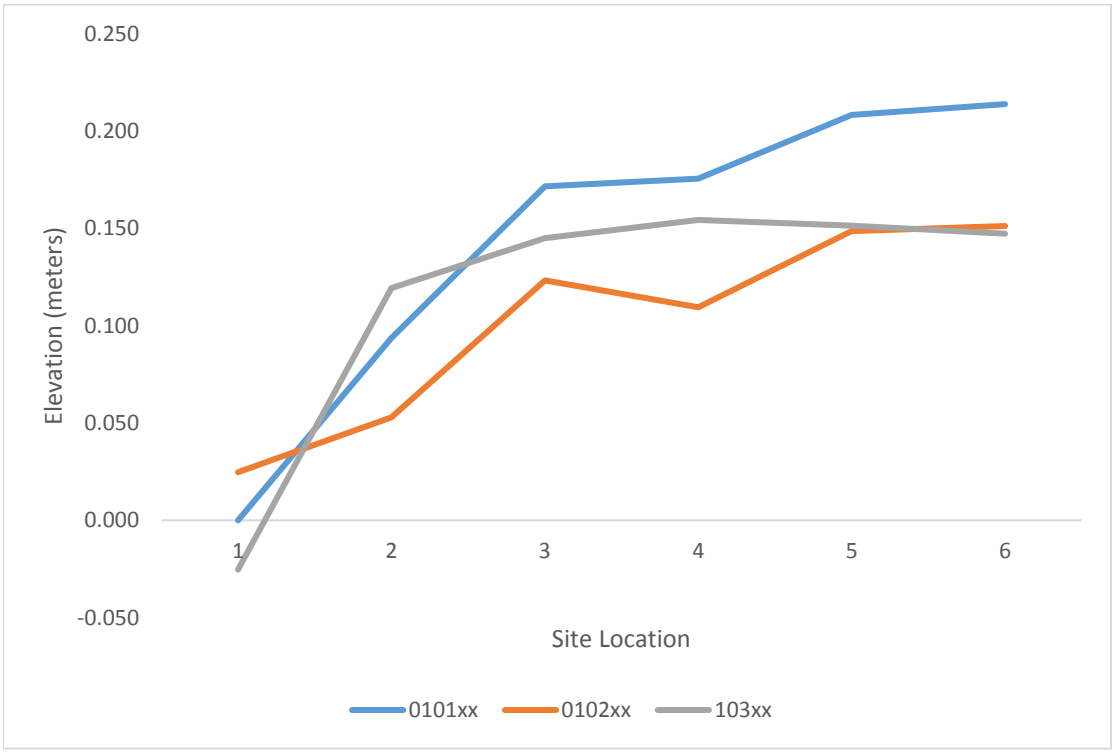


Figure 5: Elevation Profile of Site 1. Elevation measured with HOBO water level logger. All elevations relative to site 1, transect 1, location 1 (010101), which is 0 m on site 1.

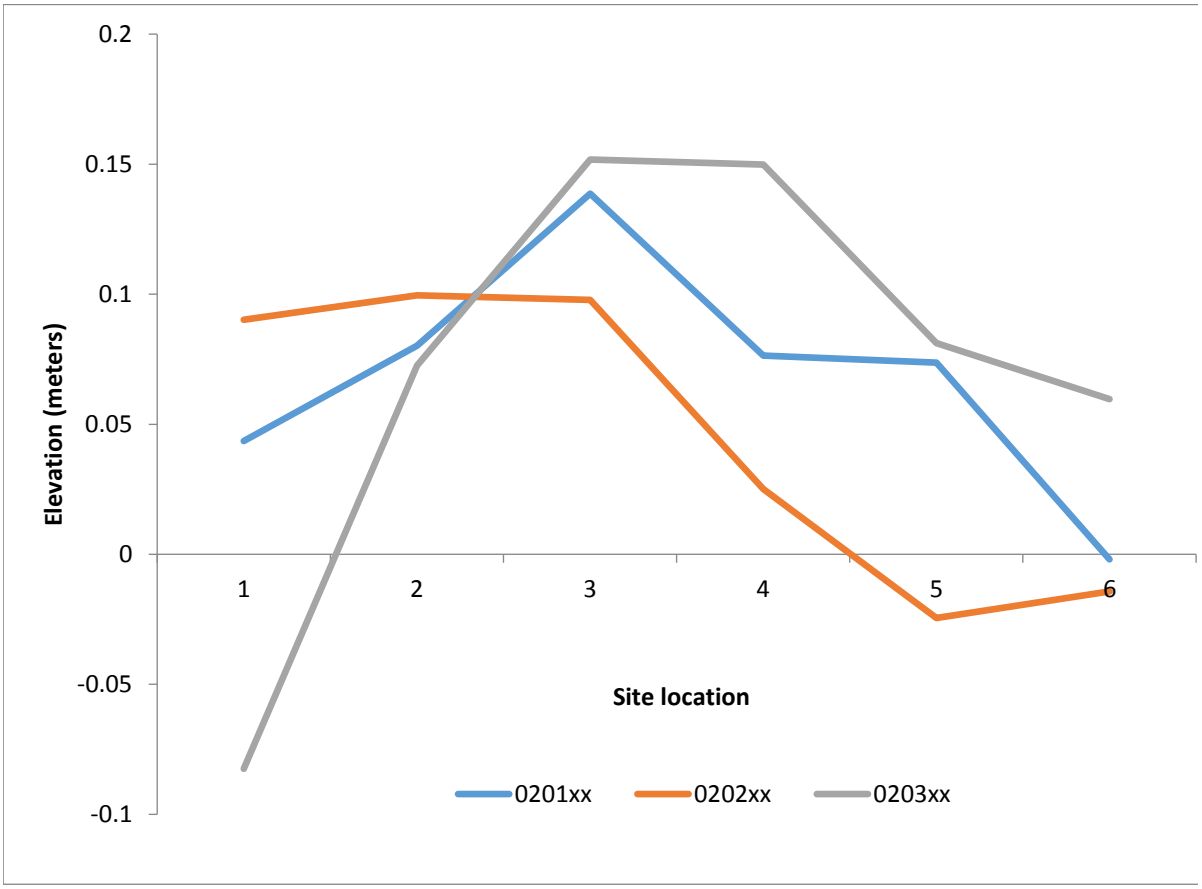


Figure 6: Elevation Profile of Site 2. Elevation measured with HOBO water level logger. All elevations relative to site 1, transect 1, location 1 (010101), which is 0 m on site 1.

Table 2 Mean Elevation for Each Transect at Site 1 and 2. Standard deviations are reported for each mean.

Site 1	0101xx Elevation (m)	N value	0102xx Elevation (m)	N value	103xx Elevation (m)	N value
xxxx01	0.000 ± 0	601	0.025 ± 0.007	1450	-0.025 ± 0.129	320
xxxx02	0.094 ± 0.013	601	0.053 ± 0.025	1450	0.119 ± 0.025	579
xxxx03	0.172 ± 0.041	601	0.123 ± 0.033	1450	0.145 ± 0.060	579
xxxx04	0.176 ± 0.043	601	0.109 ± 0.039	1450	0.154 ± 0.062	579
xxxx05	0.208 ± 0.059	601	0.149 ± 0.120	302	0.151 ± 0.086	579
xxxx06	0.214 ± 0.086	227	0.151 ± 0.120	302	0.147 ± 0.073	2887
transect mean	0.144 ± 0.083		0.102 ± 0.052		0.115 ± 0.070	
site mean	0.12 ± 0.06					

Site 2	0201xx Elevation (m)	N value	0202xx Elevation (m)	N value	0203xx Elevation (m)	N value
xxxx01	0.044 ± 0.041	2894	0.090 ± 0.047	588	-0.082 ± 0.013	579
xxxx02	0.080 ± 0.056	2894	0.100 ± 0.054	1448	0.073 ± 0.011	595
xxxx03	0.139 ± 0.076	2894	0.098 ± 0.053	1448	0.152 ± 0.039	595
xxxx04	0.076 ± 0.040	582	0.025 ± 0.033	1448	0.150 ± 0.037	595
xxxx05	0.074 ± 0.039	582	-0.024 ± .036	1448	0.081 ± 0.133	585
xxxx06	-0.002 ± 0.017	582	-0.014 ± 0.008	591	0.060 ± 0.138	585
transect mean	0.068 ±0.046		0.046 ± 0.058		0.072 ± 0.085	
Site mean	0.6 ± 0.03					

4.1.2 Redox Potential

The mean soil redox potential (Eh) for site 1 was -65.8 ±37.5 mV (Table 3). The mean soil redox potential for site 2 was -198.3 ± 30.6 mV (Table 4).

For transect 0101xx, the smallest mean Eh value occurred at location 010101, -29.2 ± 98.4 mV and the largest mean Eh value occurred at location 010103, 389.4 ±117.6 mV. The minimum Eh value occurred at location 010101, -244.5 mV, while the maximum Eh value occurred 010103, 634 mV. The mean Eh value of 1010xx was -133.6 ± 21.3 mV

For transect 0102xx, the smallest mean Eh value occurred at location 010202, -375.7 ±72.7 mV and the largest mean Eh value occurred at location 010204, 151.2 ±80.1 mV. The minimum Eh value occurred at location 010202, -556.3 mV, while the maximum Eh value occurred location 010204, 274.0 mV. The mean Eh value of 0102xx was -101.0 ± 30.5 mV.

For transect 0103xx, the smallest mean Eh value occurred at location 010302, -391.7 ±71.8 mV and the largest mean Eh value occurred at location 010304, 91.6 ±52.5. The minimum Eh value occurred at location 010301, -647.2 mV, while the maximum Eh value occurred at 010303, 181.9 mV. The mean Eh value of 0103xx was -163.6 ± 47.9 mV. Data for Site 1 can be found in Table 3.

Transect 0201xx had the smallest mean Eh value occurred at location 020106, -432.2 ± 64.1 mV and 020102, -432.0 ± 71.8 mV. The largest mean Eh occurred at location 020103, -26.1 ±112.4. The minimum Eh value occurred at location 020106, -553.9 mV. The maximum Eh value occurred at location 020103, 64.6 mV. The mean Eh value for this transect was -302.1 ± 47.9 mV.

Transect 0202xx had the smallest mean Eh value occur at location 020203, -375.5 ±125.1 mV. The largest mean Eh value occurred at location 020206, 6.9 ± 186.7 mV. The minimum Eh value occurred at location 020202, -556.9 mV, while the maximum Eh value occurred at location 020204, 338.1 mV. The mean Eh value of this site was -147.1± 28.

Transect 0203xx had a minimum mean Eh value at location 020303, -484.3 ± 121.1 mV, while the maximum Eh value was at location 020304, 136.1 ±96.0 mV. The minimum Eh value occurred at location 020306, -725.8 mV, while the maximum Eh value occurred at location 020304, 387.2 mV. The mean Eh value for this site was measured as -198.3 ± 30.6 mV. Data for Site 2 is found in table 4.

Table 3: Site 1 Eh Values. Site mean Eh values are reported as a total mean, which describes the relative redox reactions occurring at this site.

Location	Maximum Eh value (mV)	Minimum Eh value (mV)	Mean Eh (mV)	N
10101	121.5	-244.5	-29.2 ± 98.4	1413
10102	179.2	-95.5	-4.9 ± 59.6	1413
10103	634.6	-72.8	389.4 ± 117.6	1266
10104	508.2	-31.7	179.3 ± 82.0	1413
10105	-	-	-	-
10106	-	-	-	-
Transect Mean			133.6 ± 21.3	4
10201	71.2	-414.7	-158.6 ± 79.9	1441
10202	-225.4	-556.3	-375.7 ± 72.7	1441
10203	142.1	-181.9	-15.1 ± 97.0	1441
10204	274.0	26.6	151.2 ± 80.1	1441
10205	14.9	-338.7	-182.3 ± 152.5	1441
10206	99.3	-172.3	-25.6 ± 136.6	1441
Transect Mean			-101.0 ± 30.5	6
10301	-11.4	-647.2	-294.8 ± 171.0	1441
10302	-171.0	-508.4	-391.7 ± 71.8	1441
10303	181.9	-389.9	-268.5 ± 112.4	1441
10304	111.1	-7.2	91.6 ± 21.6	1441
10305	38.3	-192.8	-94.6 ± 52.5	1441
10306	50.6	-403.6	-23.5 ± 64.1	1441
Transect Mean			-163.6 ± 47.9	6

Table 4: Site 2 Eh Values. Standard deviations are reported for each mean Eh value.

Location	Maximum Eh value(mV)	Minimum Eh value (mV)	Mean Eh (mV)	N
20101	-76.1	-517.3	-332.3 ± 171.0	1098
20102	-41.6	-535.9	-432.0 ± 71.8	1098
20103	64.6	-276.4	-26.1 ± 112.4	1098
20104	-37.5	-506.7	-187.4 ± 21.6	1098
20105	-90.8	-417.6	-402.6 ± 52.5	1098
20106	-219.3	-553.9	-432.2 ± 64.1	1098
Site Mean			-302.1 ± 47.9	6
20201	15.4	-330.8	-163.2 ± 131.2	1441
20202	-256.9	-556.3	-336.4 ± 125.5	1441
20203	-185.0	-517.9	-375.5 ± 125.1	1441
20204	338.1	136.1	204.5 ± 94.7	1441
20205	-13.2	-383.1	-219.0 ± 145.2	1441
20206	39.5	-38.9	6.9 ± 186.7	1441
Site Mean			-147.1 ± 27.7	6
20301	28.5	-565.5	-256.0 ± 131.4	1441
20302	43.4	-623.3	-445.2 ± 123.9	1441
20303	66.2	-711.7	-484.3 ± 121.1	1441
20304	387.2	-152.0	136.1 ± 96.0	1441
20305	190.8	-448.8	-223.6 ± 172.7	1441
20306	146.5	-725.8	-220.6 ± 184.0	1441
Site Mean			-198.3 ± 30.6	6
Total Mean			-231.7 ± 179.2	18

4.1.2 Nitrogen Species Concentrations

Nitrate levels ranged from 0.2 mg/kg to 5.2 mg/kg. Site 1 had a mean nitrate concentration of 0.5 ± 0.3 mg/kg while site 2 had a mean of 2.3 ± 01.4 mg/kg. Site 1 had a mean ammonium concentration of 127 ± 50 mg/kg while site 2 had a mean nitrate concentration of 120 ± 41 mg/kg. Data is found in table 5.

Table 5: Nitrogen Species for all locations. Values are reported in mg/kg.

Site 1	NO_3^-	NH_4^+	Site 2	NO_3^-	NH_4^+
10101	0.6	58	20101	2.0	126
10102	0.9	80	20102	4.0	144
10103	0.8	106	20103	0.9	148
10104	0.7	170	20104	2.1	139
10105	0.5	175	20105	2.1	85.4
10106	0.5	131	20106	4.5	109
10201	0.5	194	20201	0.9	61.7
10202	0.3	79	20202	3.9	67.5
10203	0.2	59	20203	0.8	107
10204	0.2	197	20204	1.1	116
10205	0.6	136	20205	5.2	251
10206	0.4	182	20206	3.4	150
10301	0.3	182	20301	1.3	86.2
10302	0.3	179	20302	1.9	91.8
10303	0.2	95	20303	0.8	107
10304	1.2	107	20304	1.1	130
10305	0.3	117	20305	3.5	124
10306	0.5	42	20306	1.3	123
Mean	0.5 ± 0.3	127 ± 50	Mean	2.3 ± 1.4	120 ± 41

Maximum NO_3^- concentration for 0101xx was found at location 010102, 0.9 mg/kg, and the maximum NH_4^+ concentration was found at location 010105, 175 mg/kg. Minimum NO_3^-concentration was found at location 010105 and 010106, 0.5 mg/kg, and the minimum NH_4^+ concentration was found at location 010101, 58.0 mg/kg. Maximum NO_3^- concentration for transect 0102xx was found at location 010205, 0.6 mg/kg, and the maximum NH_4^+ concentration was found at location 010204, 197 mg/kg. For transect 0102xx minimum NO_3^-concentration was found at location 010203 and 010204, 0.2 mg/kg and the minimum NH_4^+ concentration was 010203, 59.2 mg/kg. For transect 0103xx maximum NO_3^- concentration was found at location 010304, 1.2 mg/kg, and the maximum NH_4^+ concentration was found at location 10301, 182 mg/kg. For transect 0103xx minimum NO_3^- concentrations were found at locations 010303, 0.2 mg/kg, and minimum concentrations of NH_4^+ was found at location 010306, 42.3 mg/kg.

For transect 0201xx maximum NO_3^- concentration was found at location 020106, 4.5 mg/kg, and maximum NH_4^+ concentration was found at location 020103, 148 mg/kg. The minimum NO_3^- concentration was found at location 020101 2.0 mg/kg, and the minimum NH_4^+ concentration was found at location 020105, 85 mg/kg. For transect 0202xx maximum NO_3^-concentration was found at location 020205, 5.2 mg/kg, and the maximum NH_4^+ concentration was also found at location 020205, 251 mg/kg. The minimum NO_3^- concentration was found at location 020203 0.8 mg/kg, and the minimum NH_4^+ concentration was found at location 020201, 62 mg/kg. For Transect 0203xx maximum NO_3^- concentration was found at location 020305, 3.5 mg/kg, and maximum NH_4^+ concentration was found at location 020304, 130 mg/kg. Minimum NO_3^-

concentration for the transect was found at location 020304, 1.1 mg/kg, and the minimum NH_4^+ concentration was found at location 020301, 86 mg/kg. All nitrogen data can be found listed in Table 5.

4.1.3 Percent Cover Species per Site

Species found at Site 1 include *Peltandra virginica*, *Polygonum sagittatum*, *Scirpus atrovirens*, *Bidens spp.*, *Typha augustifolia, Polygonum arifolium*, *Polygonum hydropiperoides*, *Impatiens capensis*, *Ludwigia palustris*, *Lathyrus palustris*, *Murdannia keisak*, *Leersia oryzoides*, *Pontedaria cordata*, unknown 1, unknown 2, *Nuphar lutea*, and *Zizania aquatica* (Table 6). Species found at site 2 include *Polygonum sagittatum*, *Bidens spp*, *Ludwigia palustris*, *Lathyrus palustris*, *Murdannia keisak*, *Leersia oryzoides Pontedaria cordata*, unknown 2, unknown oat, *Nuphar lutea*, and *Zizania aquatica* (Table 6). The species that were only found at site 1 were *Peltandra virginica, Scirpus atrovirens* Willd, *Typha augustifolia, Polygonum arifolium, Polygonum hydropiperoides, Impatiens capensis, and* Unknown 1. The species that were only found at site 2 include unknown oat.

Transect 0101xx had an annual plant percent cover mean of 14 ± 5% per plot while perennials had a mean percent cover of 44 ± 14% per plot. The highest percent cover of annuals occurred at location 010206, 22 %. The highest percent cover of perennials occurred at location 010202, 63%. Transect 0102xx had an annual plant cover mean of 7 ± 7% per plot while perennials had a mean cover of 51 ± 20 %. The highest amounts of annuals occurred at location 010203, 22% and the highest number of perennials was found at location 010204, 48%. Transect 0103xx had a mean annual

cover of 47 ± 24% per plot while perennials had a mean cover of 16 ±7%. The highest amount of annuals occurred at location 010306, 80 % and the highest amount of perennials occurred at location 010302, 27%.

Transect 0201xx had an annual cover mean of 15 ± 7 % per plot while perennials had a cover mean of 36 ± 34 % per plot percent. The highest amount of annuals occurred at location 020105, 25%, while the highest amount of perennials occurred at location 020101, 84 %. Transect 0202xx had an annual cover mean of 58 ± 29 % percent per plot while perennials had a cover mean of 6.0 ± 5 % percent per plot. The highest amount of annuals occurred at location 020206, 90%, while the highest amount of perennials occurred at location 020201, 15%. Transect 0203xx had an annual cover mean of 55 ± 19 % per plot and a perennial cover mean of 13 ± 8 % per plot. The highest number of annuals occurred at location 020304, 50%, while the highest amount of perennials occurred at location 020303, 23%. Data for percent covers of annuals and perennials are found in tables 7 and 8.

Table 6: Sites 1 and 2 Species List. Total percent cover for each species in all plots they appeared. The number of plots represents the total number of locations that each species were present.

Site 1			Site 2		
Species	**% Cover**	**Number of Plots**	**Species2**	**% Cover**	**Number of Plots**
Peltandra virginica	8	4	*Peltandra virginica*	0	0
Polygonum sagittatum	13	3	*Polygonum sagittatum*	57.5	4
Scirpus atrovirens Willd.	1	1	*Scirpus atrovirens* Willd.	0	0
Bidens spp.	44.5	9	*Bidens spp.*	47	7
Typha augustifolia	12	4	*Typha augustifolia*	0	0
Polygonum arifolium	40	11	*Polygonum arifolium*	0	0
Polygonum hydropiperoides Michx.	3	2	*Polygonum hydropiperoides*	0	0
Impatiens capensis	45.5	7	*Impatiens capensis*	0	0
Ludwigia palustris	2	1	*Ludwigia palustris*	3	1
Lathyrus palustris	39.5	9	*Lathyrus palustris*	8	2
Murdannia keisak	353	18	*Murdannia keisak*	30.52	10
Leersia oryzoides	12	4	*Leersia oryzoides*	50.02	8
Pontedaria cordata	93.5	12	*Pontedaria cordata*	36	9
Unknown 1	10.01	9	Unknown 1	0	0
Unknown 2	4	2	Unknown 2	3	1
unknown oat	0	0	unknown oat	19	4
Nuphar lutea	126.5	7	*Nuphar lutea*	206	9
Zizania aquatica	303	13	*Zizania aquatica*	660	14

Table 7: Percent Cover of Annuals and Perennials Found at Site 1. Transect means are ± 1 SD.

Location	Annual Percent Cover	Perennials Percent Covers	Annual to Perennial Ratio
10101	10	53	0.19
10102	7	63	0.11
10103	12.5	52	0.24
10104	17	40	0.43
10105	14	22	0.64
10106	22	31	0.71
Transect mean	14 ± 5	44 ± 14	
10201	0	58.5	0.10
10202	2	90	0.02
10203	22	35.01	0.63
10204	4	47.5	0.08
10205	6	27.5	0.22
10206	6	49	0.12
Transect mean	7 ± 7	51 ± 20	
10301	75	8	9.38
10302	50	27	1.85
10303	34	16	2.13
10304	27	11	2.45
10305	15	22	0.68
10306	80	9	8.89
Transect mean	47 ± 24	16 ± 7	
Mean	22 ± 23.0	37 ± 21	

Table 8: Percent Cover of Annuals and Perennials Found at Site 2.

Location	Annual Percent Cover	Perennials Percent Cover	Annual to Perennial Ratio
20101	7.5	84	0.09
20102	10	80	0.13
20103	20	30	0.67
20104	20	10	2.00
20105	25	5	5.00
20106	10	4	2.50
Transect mean	15 ± 7	36 ± 34	
20201	15	15	1.00
20202	20	6	3.33
20203	70	1	69.31
20204	75	2	37.50
20205	75	0	0.00
20206	90	10	9.00
Transect mean	58 ± 29	6 ± 5	
20301	60	6.01	9.98
20302	30	25	1.20
20303	32	22.5	1.42
20304	50	10	5.00
20305	80	10	7.99
20306	75	5	0.00
Transect mean	55 ± 19	13 ± 8	
Mean	43 ± 28	18 ± 24	

4.1.4 Species Diversity per Site

The total richness of site 1 was 17 while the total richness of site 2 was 11. The mean species diversity per site was 3.5 while the mean species diversity per plot of site 2 was 2.1. 63% were perennial and 37% were annuals. 30 % were perennials and 70% were annuals. Mean species diversity for transect 0101xx was 3.5 ± 2.0 species. Mean species diversity for transect 0102xx was 4.2 ± 2.2 species. The mean species diversity for transect 0103xx was 3.0 ± 2.0 species. Mean species diversity for transect 0201xx was 2.0 ± 0.4 species. The mean species diversity for transect 0202xx was 2.1 ± 1.7 species. The mean species diversity for transect 0203xx was 2.2 ± 1.3 species.

4.1.5 Elevation Profiles

Overall topographic shapes of sites 1 and 2 are seen in figures 5 and 6. Site 1 at all transects showed a gradual increase in elevation going towards the back marsh. Decreases in the slope of site 1 started at transect 3 for all locations and plateaued towards the back marsh. The percent change in elevation for site 1 was 112%. Site 2 showed a different topographic shape. Site 2 showed an increase in elevation towards the middle locations with a drop in elevation beyond. This change happened over 25 feet. Elevation changes for site 2 were more variable then site 1. Location 0203xx had a steep increase then transitioning to a relatively constant elevation at locations 020304, 020305, and 020306. The percent change in elevation for site 2 was 155%.

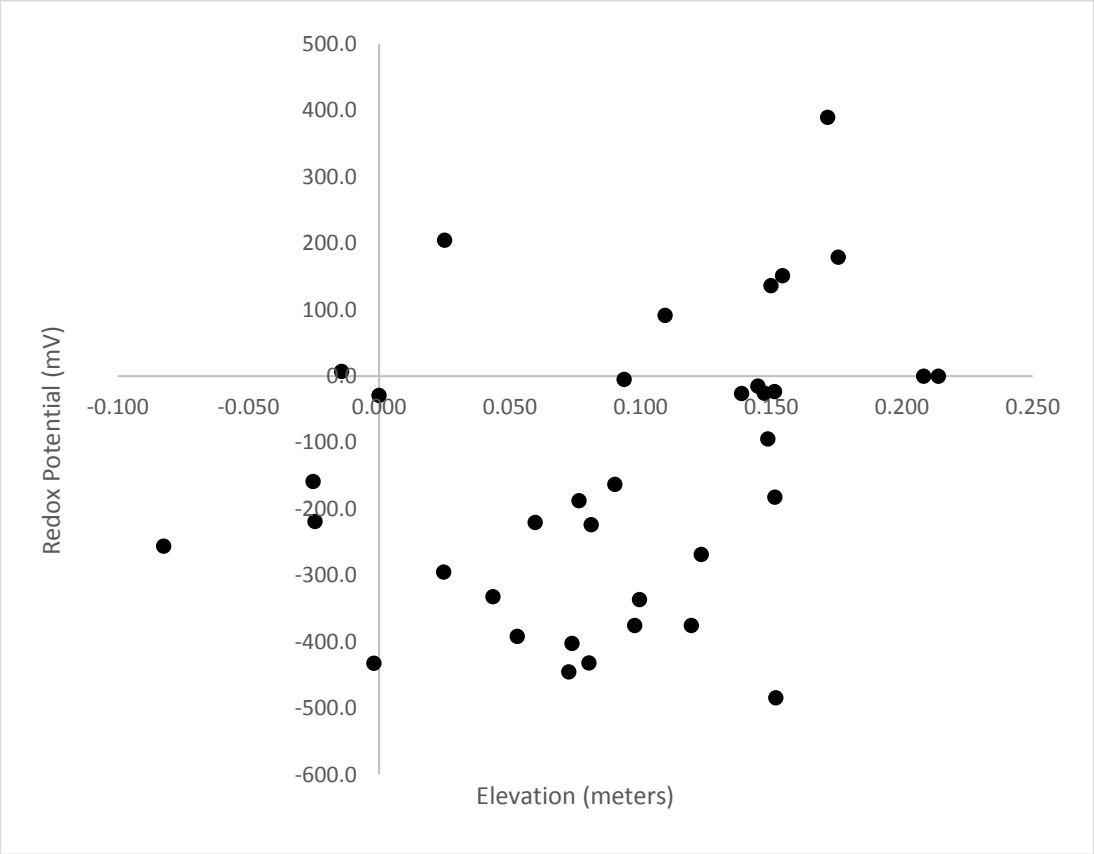


Figure 7: Mean Eh value versus Elevation. Redox potential was measured using platinum electrodes. Elevation was measured relative to a single, fixed location shown as 0 m. r=0.32 p=0.38 N=36

4.2 Redox potential versus Elevation

There was no significant correlation between mean redox potential and elevation(r= 0.32 p=0.066, N=34) (Figure 7) for all data points measured in the study. There was a correlation between elevation and maximum Eh (r=0.36 p=0.38, n=34). There was no correlation between elevation and minimum Eh (r=0.28, p=0.11, n=34).

Maximum and mean redox potential did not always peak at the same locations. At transect 0101xx, max mean and maximum Eh values peaked at location 010103, which had a relative elevation of 0.17 m. (Figure 5). The minimum Eh value was lowest at location 010101, which had a relative elevation of 0 m. The maximum elevation of this site was 0.21 m at location 010106.

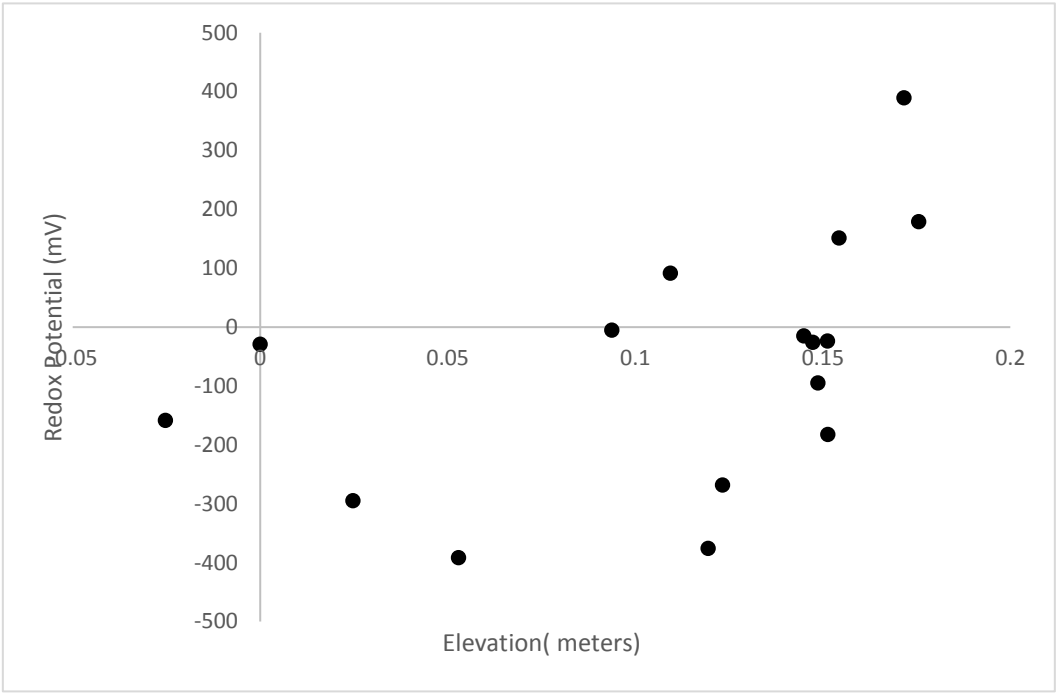
Transect 0102xx highest mean and maximum Eh value occurred at location 010204, which had relative elevation of 0.15 m. This location was also the maximum elevation for the transect. The minimum redox potential occurred at location 010202 and had a relative elevation of 0.12. The minimum elevation for this site was at location 010201 (-0.03 m). Transect 0103xx had maximum Eh value at location 010303 and at a relative elevation of 0.12 m. The max mean Eh value occurred at location 010304 and had a relative elevation of 0.11 m. The minimum Eh value occurred at location 010101 and had a relative elevation of 0.25 m. The trend observed between elevation and redox seen in Figure 8A. All data for site 1 is found in figure 8A and table 9.

Transect 0201xx had a maximum Eh and max mean value at location 020103 which had a relative elevation of 0.14. The minimum Eh value occurred at location 020106, which had the minimum elevation of 0.0 m. Transect 0202xx had a maximum

Eh and max mean Eh value at location 020204 which had a relative elevation of 0.03 m. The minimum Eh value occurred at location 020202, which had a relative elevation of 0.1 m. Transect 0203xx had a maximum and max mean Eh value at location 020304 which had a relative elevation of 0.15 m. The minimum Eh value for this transect occurred at location 020303, which had the maximum elevation for the site of 0.15 m. All data for Site 2 is found in Figure 8B and table 10.

Transect 0202xx and 0203xx did not display any Eh values that were consistent with previous findings. These transects were removed from the analysis because it is believe the electrodes degraded (addressed in discussion). After removal of transect 0202xx and 0203xx redox data, there was a significant positive correlation between mean Eh and elevation ($r=0.57$ $p=0.005$, $n=22$) (Figure 9). A significant correlation could also be seen between maximum Eh value and elevation ($r=0.53$ $p=0.011$, $n=22$) and between minimum Eh value and elevation ($r=0.59$ 0.004, $n=22$).

A. Site 1



B. Site 2.

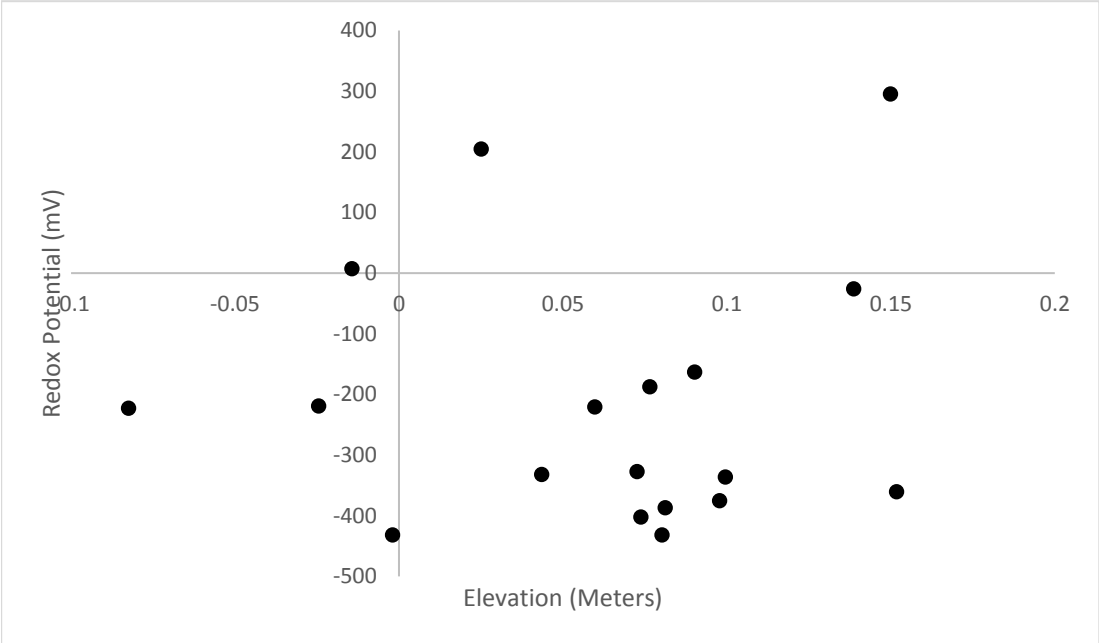


Figure 8. Mean Eh Value of Sites 1 and 2 versus Elevation. A.) Site 1 elevations and mean redox potentials. (r=0.47 p=0.07 N=16) B.) Site 2 elevations and mean redox potentials. Redox potential was measured using platinum electrodes(r=-0.029 p=0.91 N=18). Elevation was measured relative to a single, fixed location shown as 0 m.

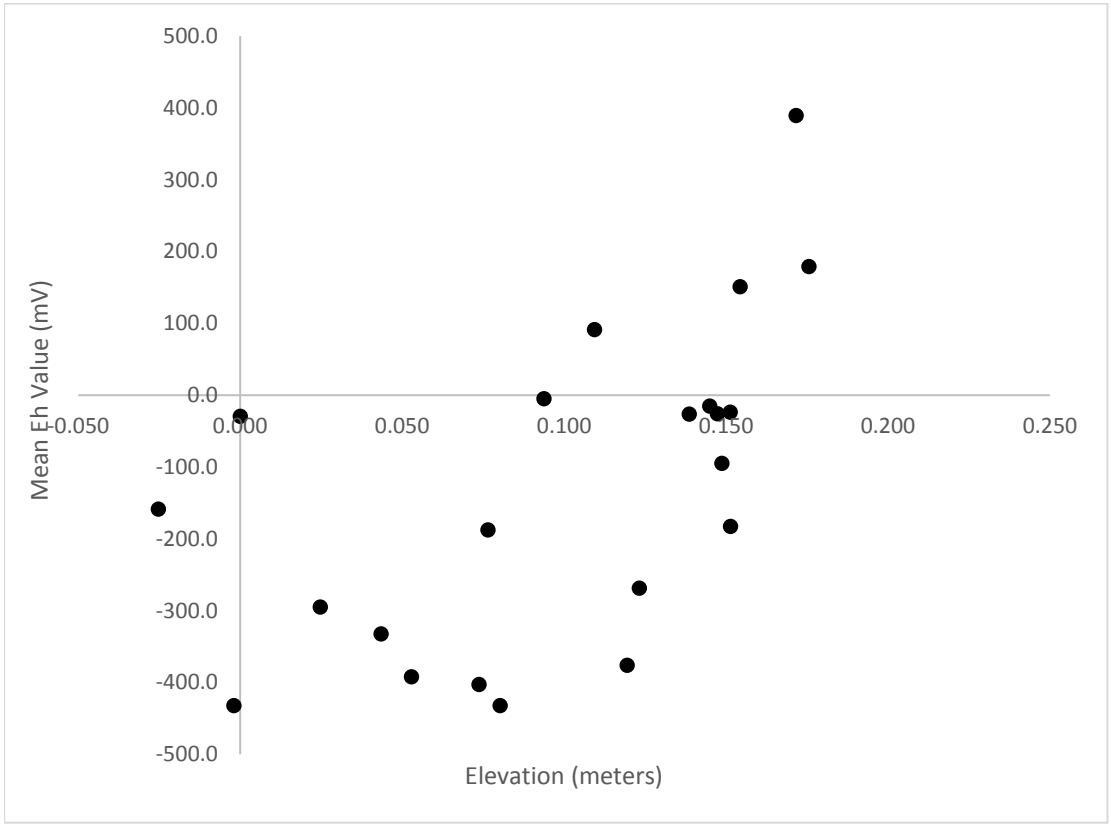


Figure 9. Elevation versus mean Eh Value. 0202xx and 0203xx Removed. Scatter plot includes sites 1 and 2 with transects 0202xx and 0203xx removed. Compare to Figure 7 that has removed transects. Redox potential was measured using platinum electrodes. Elevation was measured relative to a single, fixed location shown as 0 m. (r= r=0.57 p=0.005, n=22)

Table 9: Elevation versus Eh Values for Site 1

Location	Elevation (meters)	Maximum Eh value (mV)	Minimum Eh value (mV)	Mean Eh (mV)
10101	0.000	121.5	-244.5	-29.2
10102	0.094	179.2	-95.5	-4.9
10103	0.172	634.6	-72.8	389.4
10104	0.176	508.2	-31.7	179.3
10105	0.208	x	x	x
10106	0.214	x	x	x
10201	-0.025	71.2	-414.7	-158.6
10202	0.119	-225.4	-556.3	-375.7
10203	0.145	142.1	-181.9	-15.1
10204	0.154	274.0	26.6	151.2
10205	0.151	14.9	-338.7	-182.3
10206	0.147	99.3	-172.3	-25.6
10301	0.025	-11.4	-647.2	-294.8
10302	0.053	-171.0	-508.4	-391.7
10303	0.123	181.9	-389.9	-268.5
10304	0.109	111.1	-7.2	91.6
10305	0.149	38.3	-192.8	-94.6
10306	0.151	50.6	-403.6	-23.5

Table 10: Elevation versus Eh values for Site 2.

Location	Elevation(meters)	Maximum Eh value(mV)	Minimum Eh value (mV)	Mean Eh (mV)
20101	0.044	-76.1	-517.3	-332.3
20102	0.080	-41.6	-535.9	-432.0
20103	0.139	64.6	-276.4	-26.1
20104	0.076	-37.5	-506.7	-187.4
20105	0.074	-90.8	-417.6	-402.6
20106	-0.002	-219.3	-553.9	-432.2
20201	0.090	15.4	-330.8	-163.2
20202	0.100	-256.9	-556.3	-336.4
20203	0.098	-185.0	-517.9	-375.5
20204	0.025	338.1	136.1	204.5
20205	-0.024	-13.2	-383.1	-219.0
20206	-0.014	39.5	-38.9	6.9
20301	-0.082	28.5	-565.5	-256.0
20302	0.073	43.4	-623.3	-445.2
20303	0.152	66.2	-711.7	-484.3
20304	0.150	387.2	-152.0	136.1
20305	0.081	190.8	-448.8	-223.6
20306	0.060	146.5	-725.8	-220.6

4.3 Percent Time in Different Reduction Ranges

Both sites spent the majority of time in the high reduction and high denitrification zones. Location 01010 spent 74.5% of measured time in the highly reduce range and 25.5% of the time in the high denitrification range. 0% of time was spent in a moderate denitrification range at the aerobic threshold. Location 010102 spent 100% of measured time in a highly reduced range with 0% of this sites time ever reaching the thresholds for the denitrification range or the aerobic threshold. Location 010103 spent 0% of time in the high reduction range. 45.8 % of this locations measured time was in the high denitrification range. 52.8 % of this locations time was spent in the moderate denitrification range. 1.4 % of time was spent in the aerobic threshold zone. At location 010104, 74.3 % of this location's time was spent in the high reducing range. 23.9 % of this location's time was spent in the high denitrification range and 1.8% of the time measured was spent in the moderate denitrification range. (Figure 10).

Location 010201 spent 72.0 % of measured time in the highly reduce range and 28.0 % of time in a high denitrification range. 0% of this location's measured Eh was spent in the moderate denitrification range or the in the aerobic threshold. Location 010202 spent 100% of measured time in the highly reduced range. 0% of this locations's time was spent in the high denitrification range, moderate denitrification range, or the aerobic threshold. Location 010204 spent 0% of measured time in the highly reduced range. 8.4% of measured time was spent in the high denitrification range and 91.6% of time was spent in the moderate denitrification range. 0% of location 010204's time was spent in the aerobic threshold. Location 010205 spent 67.9% of time in the high

denitrification range and 32.1 % of time in the high denitrification range. 0% of this locations time was spent in the moderate denitrification range or in the aerobic threshold. Location 010206 spent 13.7% of time in the highly reducing range and 86.3% of time in the high denitrification zone (Figure 10).

Location 010301 spent 73.2% of time in the highly reducing zone and 25.7% of time in the high denitrification zone. 1.1% of time was spent in the moderate denitrification range while 0% of this locations time was spent in the aerobic threshold range. Location 010302 spent 100% of measured time in the highly reduced range. 0% of this location's measured Eh was spent in the moderate denitrification range or the in the aerobic threshold. Location 010303 spent 90.7% of its measured Eh time in the highly reduced range and 7.8 % of time in the moderate denitrification range. 1.5% of time was spent in the moderate denitrification range while 0% was spent in the aerobic threshold range. Location 010304 spent 0% of measured Eh value time in the highly reduced range. 010304 spent 63.6% of time in the high denitrification range. Location 010305 spent 67.3% of time in the highly reduced range and 32.7% of time in the high denitrification range. 0% of this locations measured Eh values time was spent in the moderate denitrification zone or in the aerobic threshold range (Figure 10).Location 020101 spent 97.8% of measured Eh time in the highly reducing range and 2.2% of time in the high denitrification range. This location spent 0% of time in the moderate and aerobic threshold range. Location 020102 spent 99.3% of time in the highly reducing range and 0.7% of time in the high denitrification range. This location spent 0% of time in the moderate and aerobic threshold range. Location 020103 spent 12.5% of

measured Eh time in the highly reducing range and 87.5% of time in the high denitrification range. This location spent 0% of time in the moderate and aerobic threshold range. Location 020104 spent 75.5% of time in the highly reducing range and 24.5% of time in the high denitrification range. This location spent 0% of time in the moderate and aerobic threshold ranges. Location 020105 spent 99.8% of time in the highly reducing range and 0.18% of measured time in the high denitrification range. This location spent 0% of time in the moderate and aerobic threshold range. Location 020106 spent 100% of time in the highly reducing range. 0% of this location time was spent in the high denitrification, moderate denitrification, or aerobic threshold ranges (Figure 11).

Location 020201 spent 74.9% of time in the highly reducing range and 25.1% of time in the high denitrification range. This location spent 0% of time in the moderate and aerobic threshold. Location 020202 spent 100% of measured Eh time in the highly reducing range. 0% of this location time was spent in the high denitrification, moderate denitrification, or aerobic threshold ranges. Location 020203 spent 100% of measured Eh time in the highly reducing zones. 0% of this location time was spent in the high denitrification, moderate denitrification, or aerobic threshold ranges. Location 020204 spent 0% of time in the highly reducing range and 0% of time in the high denitrification range. 100% of this locations measured Eh values time was spent in the moderate denitrification range. 0% of time was spent in the aerobic threshold. Location 020205 spent 78.3 % of measured Eh value time in the highly reducing range and 21.7% of time in the high denitrification range. This location spent 0% of time in the moderate and

aerobic threshold range. Location 020206 spent 0% of time in the highly reducing range, moderate denitrification zone, and the aerobic threshold ranges. 100% of this locations time was spent in the high denitrification range (Figure 11).

Location 020301 spent 89.2% of time in the highly reducing range. 10.8% of time was spent in the high denitrification range. 0% of time was spent in the moderate denitrification and aerobic threshold range. Location 020302 spent 100% of time in the highly reducing range and 0% of time in all other zones. Location 020303 spent 99.5% of time in the highly reducing range. 0.5% of time was spent in the high denitrification range. 0% of time was spent in the moderate denitrification and aerobic threshold range. Location 020304 spent 0% of time in the highly reducing range and high denitrification range. 0% of time was spent in the moderate denitrification and aerobic threshold range. Location 020304 spent 0% of time in the highly reducing range and high denitrification range. 96.6% and 3.4% of time were spent in the moderate denitrification and aerobic threshold range, respectively. Location 020305 spent 98.8% of time in the highly reducing range and 1.8% of time in the high denitrification range. 0% of time was spent in the moderate denitrification and aerobic threshold range. At location 020306 73.9% and 26.1% of time was spent in the highly reducing range and the high denitrification range, respectively. 0% of time was spent in the moderate denitrification and aerobic threshold range (Figure 11).

A. Site 1

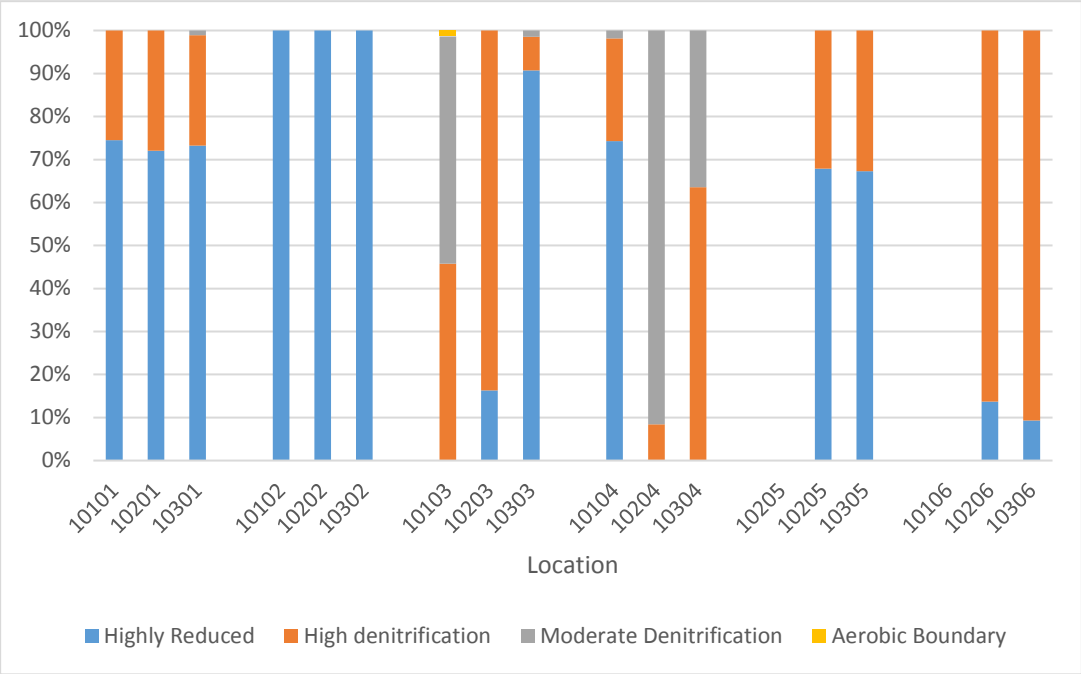


Figure 10: Redox Ranges Site 1. Locations are arranged from 1st to 6th location for each transect at a site. For example, first three bars are 1st location of each transect at a site.

B. Site 2

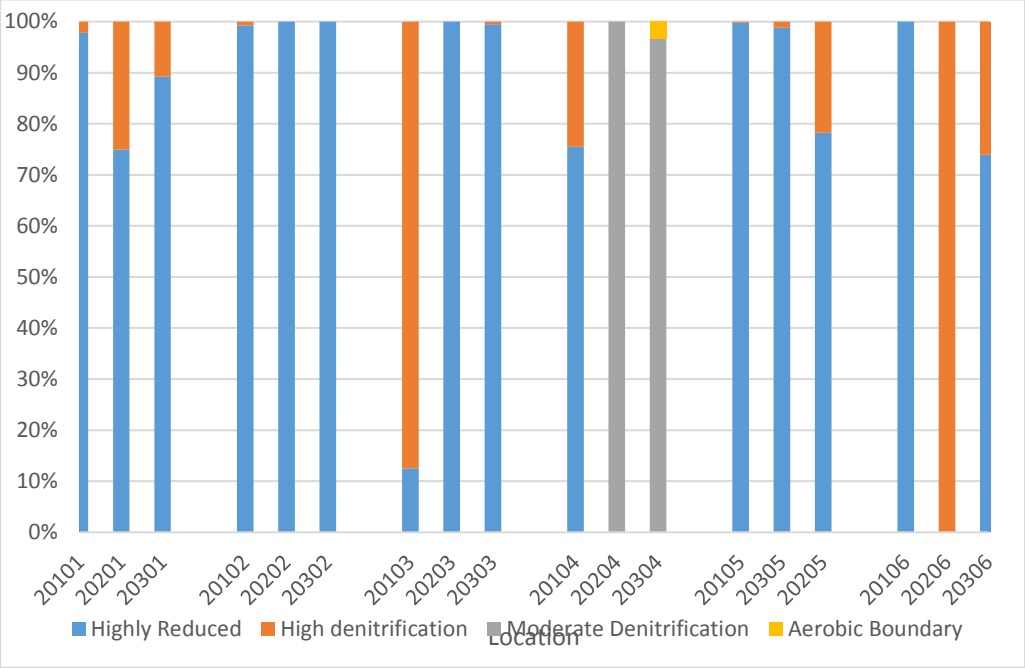


Figure 11: Redox Ranges Site 2. Locations are arranged from 1st to 6th location for each transect at a site. For example, first three bars are 1st location of each transect at a site.

4.4 Elevation versus Species Diversity

There was a positive correlation between relative elevation and species diversity for all sampled locations ($r=0.65$ $p<0.001$, $n=36$) (Figure 12). Max species diversity did not always correlate to maximum elevation of the transect. However, maximum species diversity is seen in the higher elevation areas. Species diversity at site 1 was higher in high elevation areas and low in the lower elevation areas. Species diversity peaked when elevations neared their max for the site. At transect 0101xx species diversity was highest at location 010104 (0.18 m) and was lowest at location 010102 (0.09 m). At transect 0102xx maximum species diversity occurred at location 010203 while the minimum species diversity occurred at the minimum elevation at 010201 (-0.03 m). Transect 0103xx had a maximum species diversity at location 010305 (0.15), which was the maximum elevation for the site. The minimum species diversity occurred at location 010101 (0.03 m), which was the lowest elevation for the site. Site 2 showed similar trends. At transect 0201xx, maximum species diversity was found at location 020103 (0.15), which was the maximum elevation for this transect. The minimum species diversity for this location was found at location 020101 (-0.08 m), which was the minimum elevation for Site 2. Transect 0202xx had a maximum species diversity at location 020201 (0.09 m). The minimum species diversity occurred at locations 020203, 020304, and 020305. Location 020203 was the maximum elevation for this location.

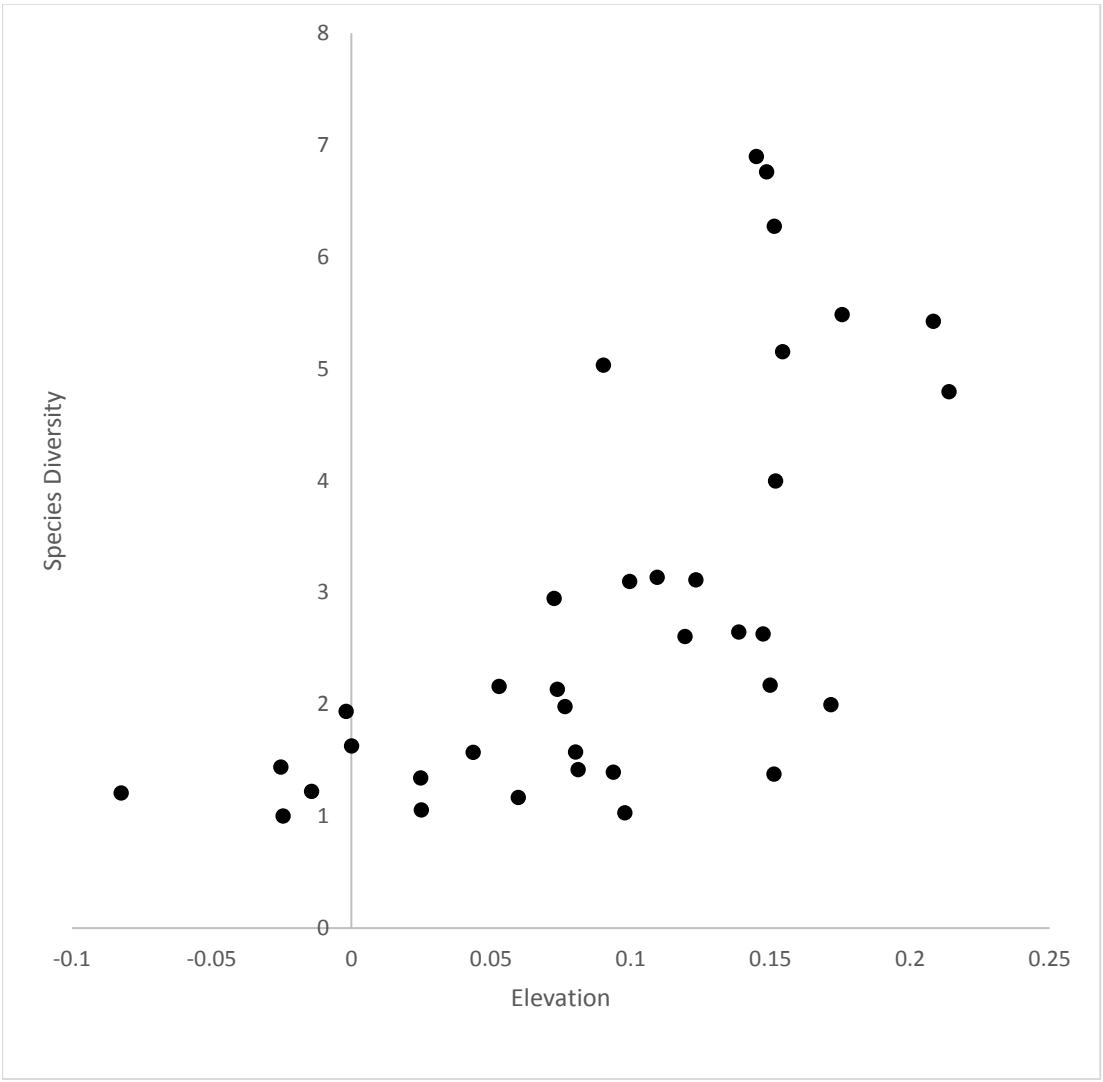


Figure 12: Diversity versus Elevation. r= 0.65 0<0.001 N= 36. Elevation relative to reference point 0 m. Species Diversity is 1/D from Simpson-Wiener Biodiversity Index

4.5 Species Diversity and Redox Potential

There was no significant correlation between species diversity and maximum, minimum, or mean Eh value. Correlation was tested when electrodes from transects 0202xx and 0203x were removed from the analysis. There was a significant correlation between minimum Eh value and species diversity ($r= 0.49$ $p=0.023$ $N=22$)(Figure 13). There were still no significant correlations between maximum Eh and mean Eh.

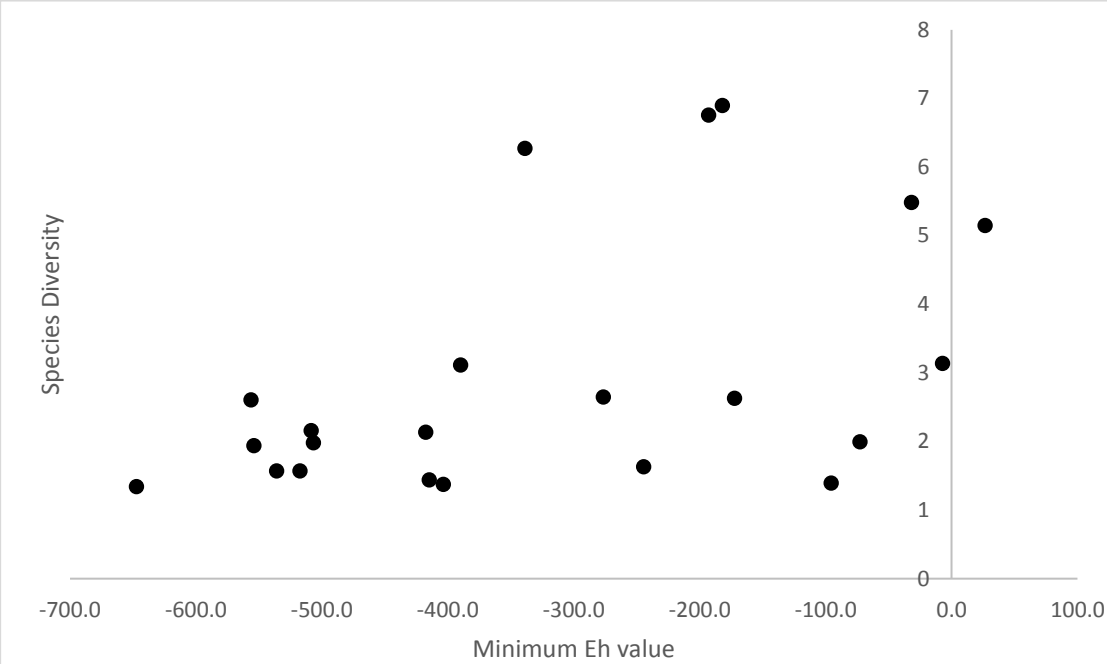


Figure 13: Minimum Eh versus Species Diversity. Transects 0202xx and 0203xx were removed from the analysis. r= 0.49 p=0.023 N=22. Minimum Eh value was recorded by taking the minimum value that was recorded at locations. Species Diversity was measured as 1/D using Simpson-Wiener Biodiveresity Index.

4.6 Nitrogen species versus Elevation

Site 1 had a mean nitrate concentration of 0.051 ± 0.03 mg/kg while site 2 had a mean nitrate concentration of 0.2 ± 0.14 mg/kg (Table 11 and 12). Site 1 had a greater mean elevation than site 2

Nitrate levels decreased as elevation increased (Figure 14, r= 0.46 p=0.05, N=36). Nitrate levels were highest at -0.2 m (0.52 mg/kg) and were lowest at 0.15 m (0.18 mg/kg). At site 1 the maximum nitrate concentration occurred at location 010304 (0.11 m) and was 0.12 mg/kg. The minimum nitrate concentration for site 1 was found at location 010204 (0.154 m) and was 0.018 mg/kg. There was no correlation between NH_4^+ concentrations and elevation.

4.7 Nitrogen Species versus Species Diversity

Nitrate levels decreased with increased species diversity of plots (Figure 15); (r=-0.40 p=0.015, N=36). Site 1, which had a higher species diversity than site 2, had a lower mean nitrate concentration (Table 9 and 10). The maximum nitrate levels were found at location 020205. The species diversity for 020205 was 1 and was dominated by *Z. aquatica*. The minimum nitrate levels occurred at locations 010203 and 010204. The nitrate levels at this location were 0.19 mg/kg and 0.18 mg/kg, respectively. The species diversity of these locations was 6.9 and 5.2 respectively.

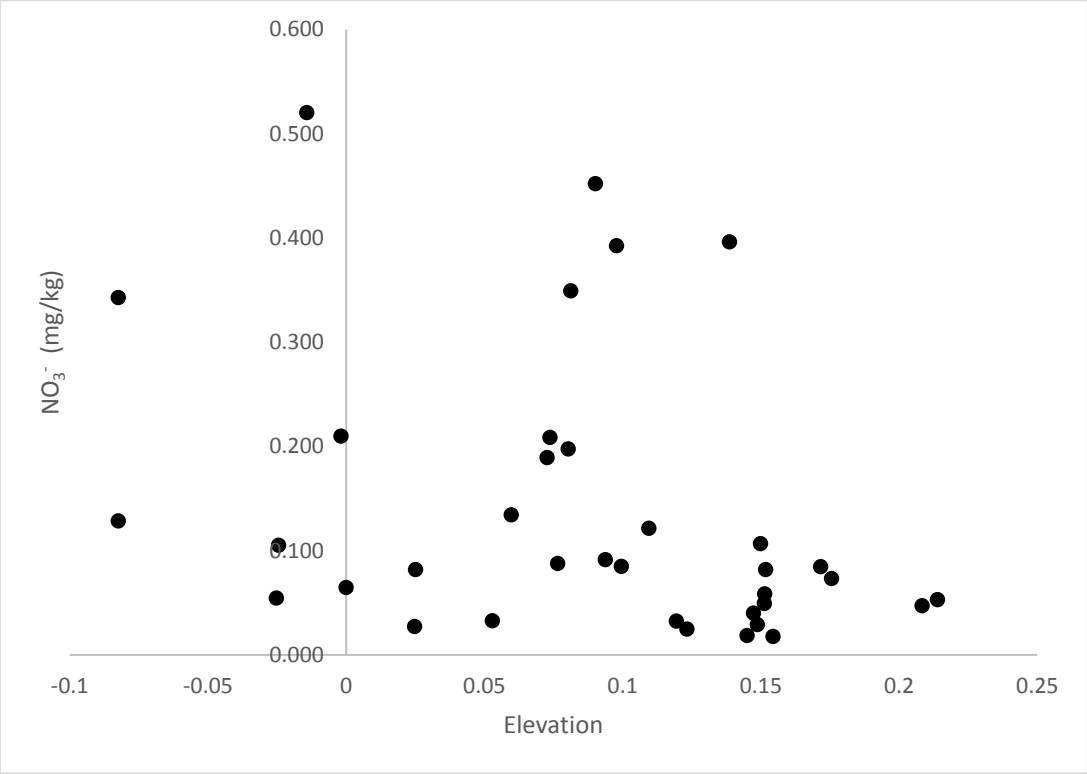


Figure 14: Elevation versus Nitrate Concentrations (mg/kg). R= 0.46. Elevation was measured relative to a single, fixed location shown as 0 m. Nitrate levels were measured using total N extraction.

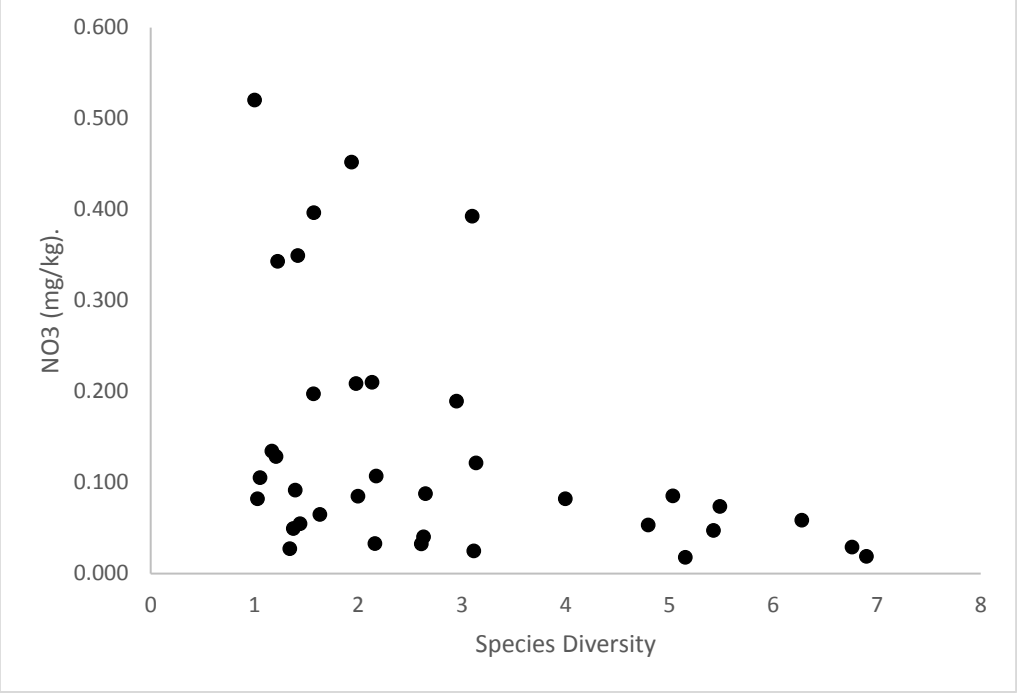


Figure 15: Species Diversity versus Nitrate Concentrations (mg/kg). . Species Diversity was measured as 1/D using Simpson-Wiener Biodiveresity Index. NO_3^- was calculated from total N extraction. r=-0.40 p=0.015 N=36.

Table 11: Concentration of N species in mg/kg for Site 1.

Location	Elevation (meters)	NO_3^- (mg/kg)	NH_4^+ (mg/kg)	
10101	0.000	0.065	5.788	
10102	0.094	0.092	8.030	
10103	0.172	0.085	10.574	
10104	0.176	0.073	16.961	
10105	0.208	0.047	17.454	
10106	0.214	0.053	13.181	
10201	-0.025	0.055	19.372	
10202	0.119	0.032	7.923	
10203	0.145	0.019	5.917	
10204	0.154	0.018	19.728	
10205	0.151	0.058	13.560	
10206	0.147	0.040	18.179	
10301	0.025	0.027	18.175	
10302	0.053	0.033	17.912	
10303	0.123	0.025	9.523	
10304	0.109	0.121	10.725	
10305	0.149	0.029	11.717	
10306	0.151	0.049	4.227	
		Mean	Mean	N
		0.051 ± 0.03	12.72 ± 5.2	18.0

Table 12: Concentration of N species in mg/kg for Site 2.

Location	Elevation (meters)	NO_3^-(mg/kg)	NH_4^+ (mg/kg)	
20101	0.044	0.2	12.6	
20102	0.080	0.396	14.382	
20103	0.139	0.088	14.769	
20104	0.076	0.209	13.845	
20105	0.074	0.210	8.539	
20106	-0.002	0.452	10.845	
20201	0.090	0.085	6.172	
20202	0.100	0.393	6.746	
20203	0.098	0.082	10.709	
20204	0.025	0.105	11.564	
20205	-0.024	0.520	25.108	
20206	-0.014	0.343	15.026	
20301	-0.082	0.1	8.6	
20302	0.073	0.2	9.2	
20303	0.152	0.082	10.709	
20304	0.150	0.1	13.0	
20305	0.081	0.349	12.386	
20306	0.060	0.134	12.331	
		Mean	Mean	N
		0.2 ± 0.14	12.0 ± 4.0	18

4.8 Annual and Perennial Cover

4.8.1 Annuals and Perennial Cover versus Elevation

There was a negative correlation between elevation and total annuals organisms for all locations (r=-0.49 p=0.003 N= 36) (Figure 16). No significant trend was seen between elevation and perennial cover (r=0.33 p=0.06 N=36). A significant negative correlation was found between elevation and the ratio of annuals to perennials (r=-0.50 p= 0.03 N=36) (Figure 17). The highest ratio of annuals to perennials occurred at the minimum elevation (-0.08 m). The lowest ratios of annuals to perennials did not occur at just one location due to the lack of annuals being found at some sights. Elevations that had zero or trace levels of annuals include elevations ranging from -0.014 m to 0.12 m. Site 1, which had a higher mean elevation had a lower percent cover of annuals at all locations (22 percent annual cover per location). Site 2, which had a lower mean elevation had a higher total number of annuals across all locations, 42% cover per location of annuals.

4.8.2 Annual and Perennial Cover versus Species Diversity

There was a negative correlation between species diversity and number of annuals / location (r=-0.49 p=0.01 N=36) (Figure 18). No significant correlations were found between species diversity and perennials or the ratio of annuals to perennials. However, two outliers were identified used SPSS software. Two outliers were removed from the ratio of annuals to perennials data set and a negative correlation was seen (r=-0.41 p=0.016, N=36) (Figure 19). When comparing annuals cover to other variables,

annual cover heavily influenced when there is a lack of annuals. There was a significant negative correlation between annual percent cover per plot and perennial percent cover per plot (-0.36 p=0.006, N=36) (Figure 20). Refer to tables 13 and 14 for data on annuals and perennials.

4.8.3 Appearance of *A. virginica*

A. virginica was not found in any sampled locations at sites 1 or 2. No correlations between wetland physiochemical properties and *A. virginica* could be calculated.

4.8.4 Annual and Perennial Cover versus Eh values

There was no significant correlation between maximum Eh and annual cover (r=-0.107 p= 0.55 N=34). There was no significant correlation between mean Eh and annual cover (r= 0.35 p=0.85 N=34) There was no significant correlation between minimum Eh and annual cover (r=0.07, p=0.7, N=34). There was no significant correlation between maximum Eh and perennial cover (r=0.178 p=0.32 N=34). There was no significant correlation between mean Eh and perennial cover (r=0.157 p=0.38 N=34). There was no significant correlation between minimum Eh and perennial cover (r=0.16 p=0.38 N=34).

4.12.4 Annual and Perennial Cover versus N species.

There was no significant correlation between annual cover and NO_3^- (r=0.166 p=0.34 N=36). There was no significant correlation between annual cover and NH_4^+ (0.049 p=0.78 N=36). There was no significant correlation between perennial and NO_3^-

(r=-0.044 p=0.8 N=36) There was no significant correlation between perennial and NH_4^+ (r=0.44 p=0.8 N=36).

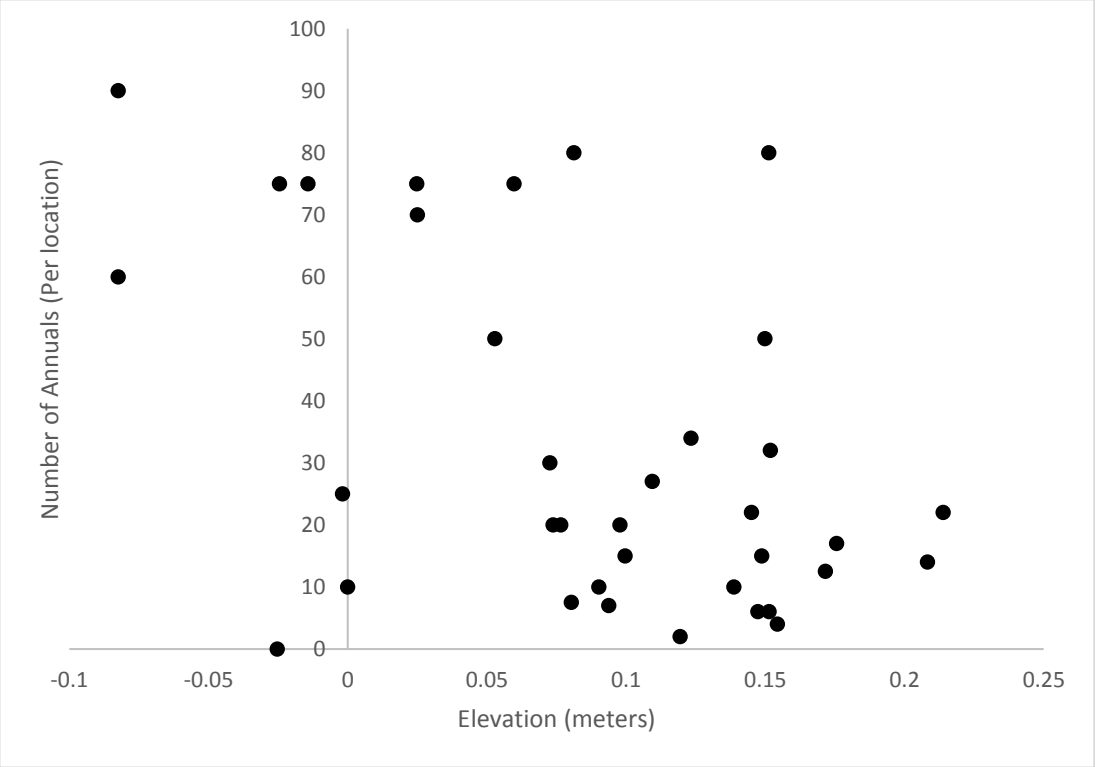


Figure 16: Elevation versus Percent Cover of Annuals (r=-0.49 p=0.003 N=36). Elevation is relative to location 010101, 0 m.

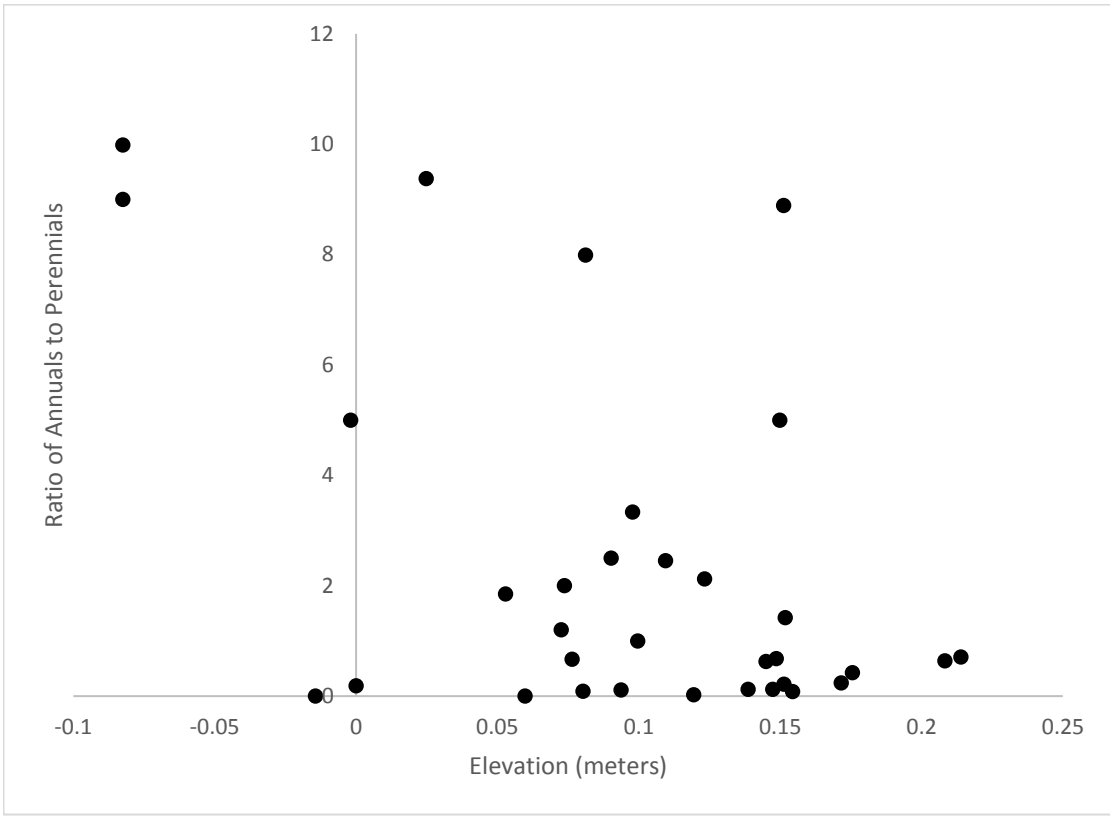


Figure 17: Elevation versus the Ratio of Annuals to Perennials (r=-0.50 p=0.03 N=36).

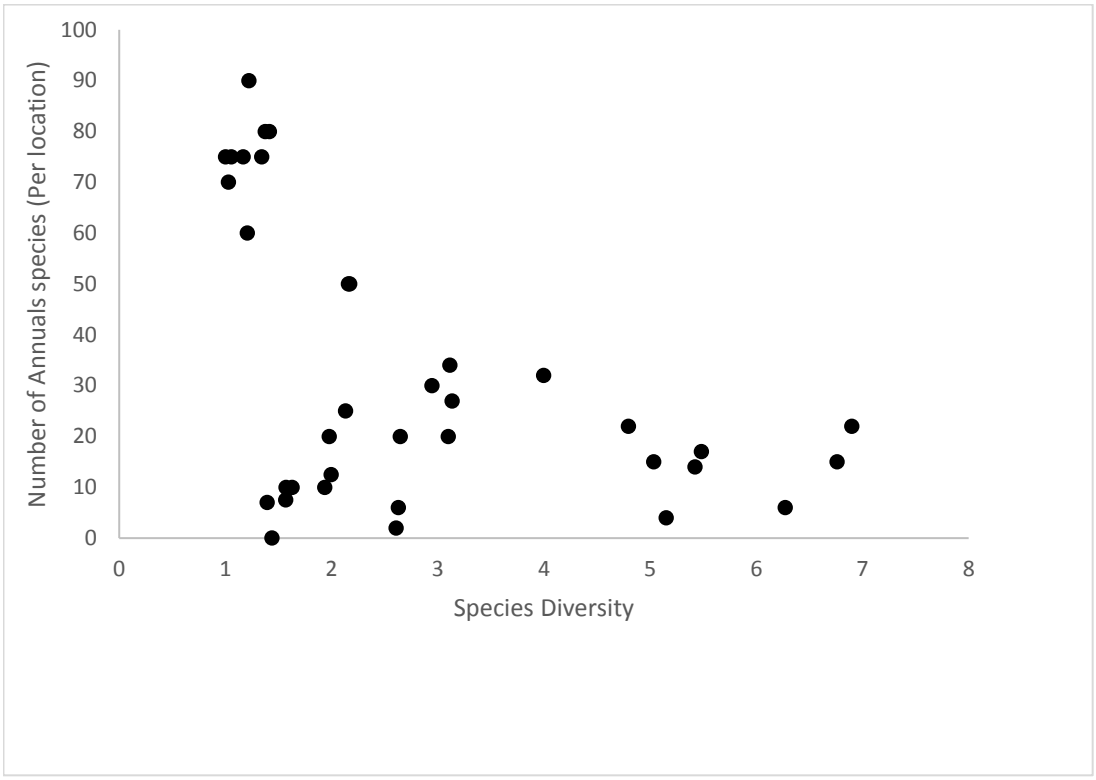


Figure 18: Species Diversity versus the Percent Cover of Annual/ Perennials (r=-0.49 p=0.01 N=36).

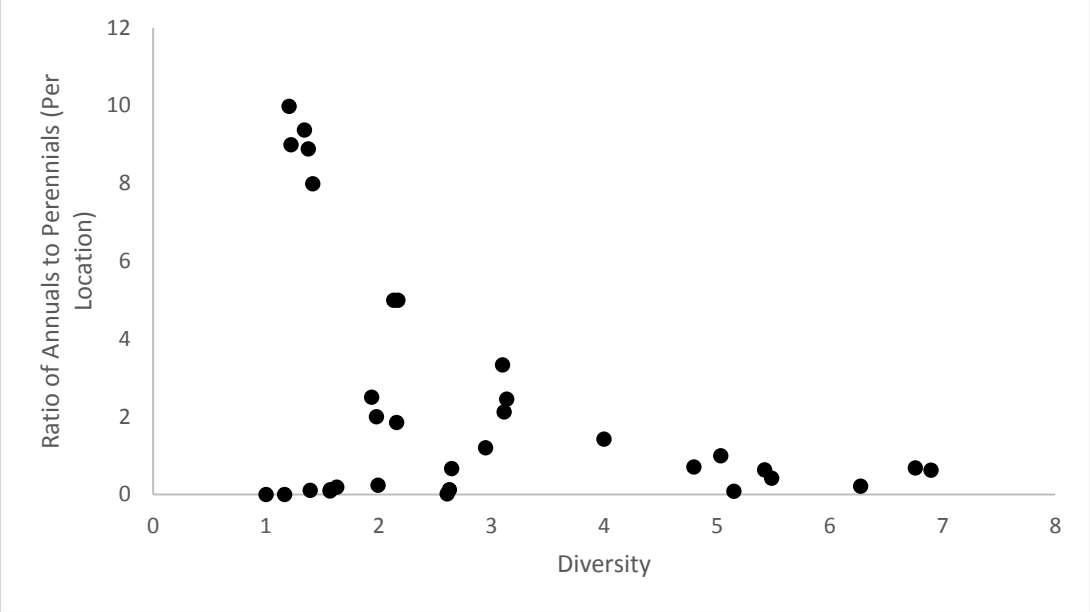


Figure 19: Species Diversity versus the Ratio of Annuals/Perennials (r=-0.41 p=0.016 N=36).

Table 13: Elevation and Annual and Perennial Cover at Site 1.

Location	Elevation	Diversity index	Total Annuals at Location	Total Perennials at Location	Ratio of Annuals to Perennials
10101	0.00	1.63	10.00	53.00	0.19
10102	0.09	1.39	7.00	63.00	0.11
10103	0.17	1.99	12.50	52.00	0.24
10104	0.18	5.48	17.00	40.00	0.43
10105	0.21	5.42	14.00	22.00	0.64
10106	0.21	4.79	22.00	31.00	0.71
10201	-0.03	1.44	0.00	58.50	
10202	0.12	2.61	2.00	90.00	0.02
10203	0.14	6.90	22.00	35.01	0.63
10204	0.15	5.15	4.00	47.50	0.08
10205	0.15	6.27	6.00	27.50	0.22
10206	0.15	2.63	6.00	49.00	0.12
10301	0.02	1.34	75.00	8.00	9.38
10302	0.05	2.16	50.00	27.00	1.85
10303	0.12	3.11	34.00	16.00	2.13
10304	0.11	3.14	27.00	11.00	2.45
10305	0.15	6.76	15.00	22.00	0.68
10306	0.15	1.37	80.00	9.00	8.89

Table 14: Elevation and Annual and Perennial Cover at Site.2.

Location	Elevation	Diversity index	Total Annuals at Location	Total Perennials at Location	Ratio of Annuals to Perennials
20101	0.08	1.57	7.50	84.00	0.09
20102	0.14	1.57	10.00	80.00	0.13
20103	0.08	2.65	20.00	30.00	0.67
20104	0.07	1.98	20.00	10.00	2.00
20105	0.00	2.13	25.00	5.00	5.00
20106	0.09	1.94	10.00	4.00	2.50
20201	0.10	5.03	15.00	15.00	1.00
20202	0.10	3.10	20.00	6.00	3.33
20203	0.03	1.03	70.00	1.01	3.33
20204	-0.02	1.05	75.00	2.00	69.31
20205	-0.01	1.00	75.00	0.00	0.00
20206	-0.08	1.22	90.00	10.00	9.00
20301	-0.08	1.21	60.00	6.01	9.98
20302	0.07	2.95	30.00	25.00	1.20
20303	0.15	4.00	32.00	22.50	1.42
20304	0.15	2.17	50.00	10.00	5.00
20305	0.08	1.42	80.00	10.01	7.99
20306	0.06	1.17	75.00	5.01	0.00

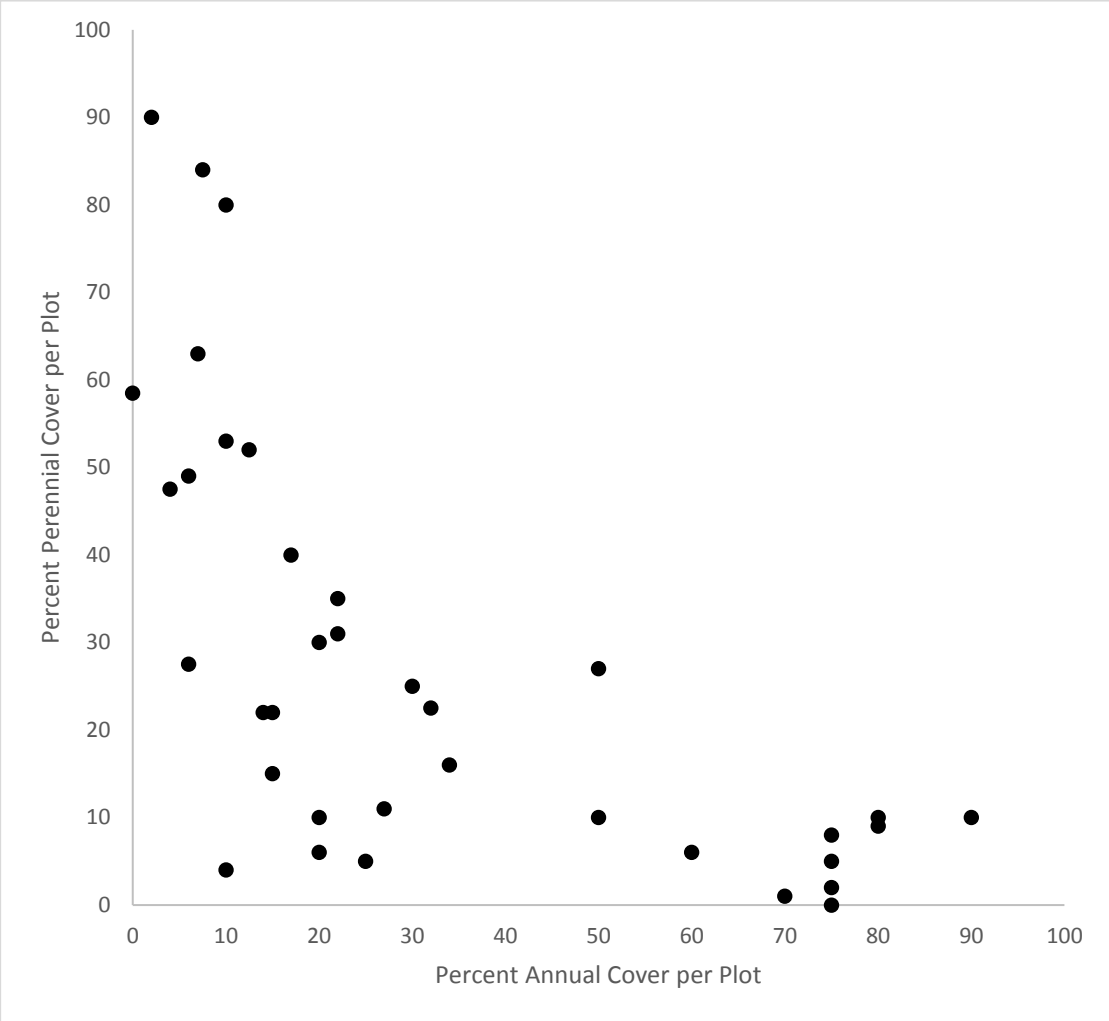


Figure 20: Percent Cover Annuals versus Perennials. Percent cover measured from the percent cover of individuals at the location (r=-0.36 p= 0.06 N=36)

5. Discussion

5.1 Elevation

Between sites 1 and 2 the elevation change was 30 cm. The two sites displayed different topographic shapes, both are typical of tidal freshwater wetlands. Site 1 displayed a steep increase in elevation over the first two locations (01xx01 and 01xx02) at each transect. The elevation change decreased towards the back marsh and remained relatively flat. Transect 0201xx and 0203xx at site 2 had a steep increase in elevation at the first two locations (02xx01 and 02xx02). Transect 0202xx did not show a steep increase in elevation at the first two transects, but rather it displayed a flat decline in elevation towards 020203. All three transects reached a maximum elevation at location 02xx03. After the elevation peak, elevations decreased drastically towards the back marsh.

The topographic cross sections recorded in this study are typical cross sections found in tidal freshwater wetlands (Mitsch and Gosselink 2007) The two different topographic shapes of each site are the foundation of any changes in the chemical and biological environment that are observed over the micro elevation changes.

Elevation was shown to play an important role in reduction potentials, nitrate levels and species diversity in this study. This is the first study to show that micro-elevation changes over relatively small distances have a significant effect on wetland function. Elevation is the driver for tidal inundation, redox potential variation, nutrient availability, and species composition (Wetlands 2000; Hopfenberger et al. 2007 and

2009). Wetland elevation changes as small as 30 cm had dramatic effects on wetland cycles. Locations that were only 15 cm different from one another had dramatically different redox potentials and in some cases species diversity (Figure 3, Figure 5, Table 5). Commonly, we view marshes as being flat on the large scale, but the elevation differences in these wetlands created microhabitats that have various physiochemical conditions.

5.2 Reducing Potential of Soil

Overall, redox potential was measured in the high denitrification to moderate denitrification range at all sites. Soils remained in a high reduction range for majority of the measured time. Redox ranges measured were between high reduction to high denitrification range and occasionally reaching into moderate denitrification and aerobic threshold.

The high percentage of time spent in high reduction and high denitrification ranges is an indication that soils remained saturated with water and were poorly drained. The mean Eh across all locations was -154 mV, which falls into the high denitrification range. While soils occasionally reached into the aerobic threshold soils did not reach an oxidized state, which would have been indicated by an Eh reading above 400 mV. Aerobic conditions were not established for extended periods of time therefor oxidation of chemical species could not occur

5.3 Redox versus Elevation

When redox values and elevation were first compared, there were no significant correlations between mean Eh and minimum Eh. Maximum Eh and elevation did have a significant correlation ($r= 0.36$ $p=0.38$ $N=34$). The insignificant correlations could be due to the degradation of electrodes as the study went on. Constructed Pt electrodes are known to be efficient ways to measure redox potentials in submerged soils, however maintenance and retesting in Zobell's solution is required. Maintenance was done after measurements of transects 0101xx, 0102xx, 0103xx, and 0201xx but not done before 0202xx or 0203xx. The time frame for electrode measurements did not permit proper maintenance before each use on the later transects. For the purposes of our statistical analyses, all redox potentials remained in the initial analyses. Correlations were tested when transects 0202xx and 0203xx were removed.

When transects 0202xx and 0203xx were removed from the analysis, there was a significant positive correlation between elevation and all redox parameters. As elevation increased on our sites, the redox potential of the soils increased. This finding is significant to this study because it shows that over micro elevation changes (30 cm) redox potential is impacted. This may be created by time of inundation or water level at different elevations. At higher locations in the marsh, the time of inundation is shorter and amount of inundation is less than lower elevations locations.

Due to tidal inundation cycles being controlled by many sub-factors, elevation may not be the sole driver of Eh values in the wetland. Although there was a significant

correlation between redox and elevation, other physical factors that affect hydrology could have an impact on redox potential. For example, slope could lead to surface depressions which may pool water leaving soils saturated for longer periods of time.

5.4 Nitrate Concentrations

Low levels of nitrate were present across all sites. Standard fertilization experiments range from 1 to 60 mg/kg with 1 mg/kg representing low levels of nitrate and 60 mg/kg representing very high levels (Kirk 2005). The nitrate levels in wetland soils observed in this study did not exceed 0.5 mg/kg. This might be explained by the consistently low redox potentials that were present in the wetland soils observed. In wetlands, NO_3^- entering the system is denitrified before it reaches a sufficiently reduced environment. Soils were almost always in a highly reduced state and recovery to more oxidized states are slow. This halted the accumulation of NO_3^- in the soil (Buresh and Patrick, 1981). Low NO_3^- levels across both sites in this study are consistent with these previous findings.

NH_4^+ accumulates in soil, which lack oxygen (Mengel and Kirkby 1987). The high levels of ammonium in the wetland soil are consistent with the parameters for ammonification and nitrification not being met, which is sufficient O_2. Low redox potential show that the soils are in an oxygen depleted state for large percentages of time. The highly reducing soils at both sites likely stop NH4 nitrification and thus NH4 accumulates in soil solution. The mean for NH_4^+ measured for sites 1 and 2 were both 124 mg/kg. Means that are similar could suggest NH_4^+ in the soil is accumulating evenly

throughout the soil. The finite soil surfaces spaces are completely filled with NH_4^+ at both sites due to the soils specific cation exchange capacity (CEC). As nitrate reduction begins, NH_4^+ begins to accumulate in the soil (Mitsch and Gosselink 2007). As lower reducing conditions are met, NH_4^+ concentrations being to level off. This is an indication that the soil has filled all available CEC cites and NH_4^+ can no longer accumulate.

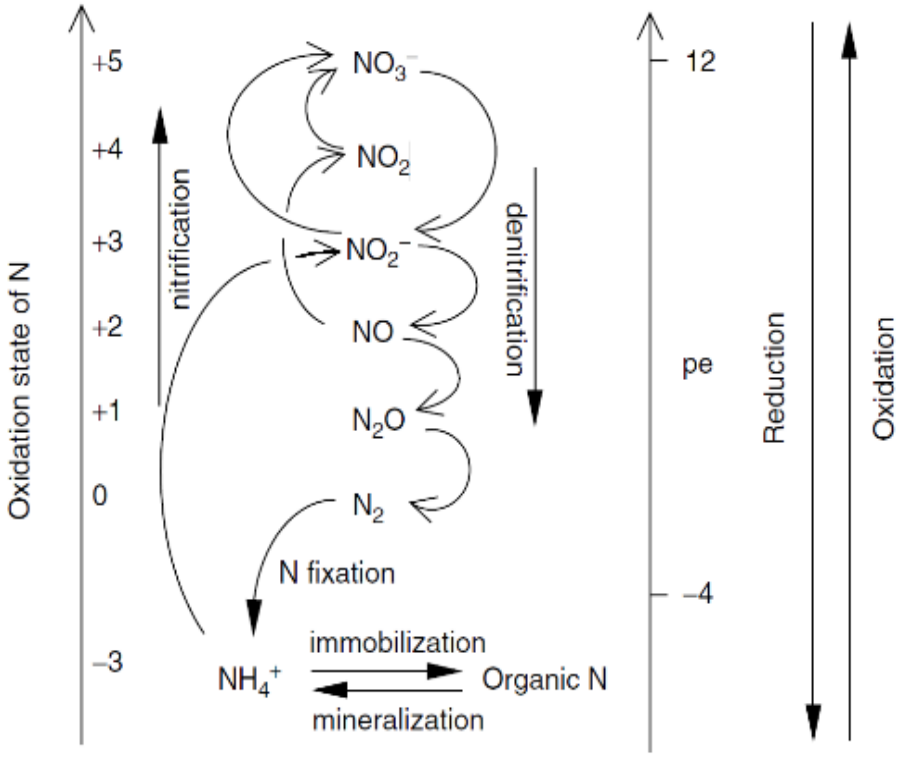


Figure 21: Soil Nitrogen Cycle and Oxidation and Reduction States NO_3^- is reduced to N_2. N_2 is then fixated to NH_4^+ in the absence of oxygen. NH_4^+, which requires oxygen to under go nitrification, accumulates in submerged soils.

From Kirk 2005

Rhizosphere processes in wetland soils are crucial in the uptake of NO_3^- in wetland plants. Knonzucker et al. (1999, 2000) found that rice grown in anaerobic conditions became extremely efficient in absorption of NO_3^- formed by nitrification in the rhizosphere. This became the primary source of N for the plants. The rate of NO_3^- absorption depends on the rate of formation and loss in the rhizosphere (Kirk 2005). The low levels of NO_3^- found at sites 1 and 2 are consistent with previous studies. Roots transport oxygen through aerenchyma tissue and allow for the nitrification of NH_4^+ to NO_3^-. The nitrogen which is absorbed is local to plant rhizosphere and is not distributed through the overall bulk soil.

NO_3^- is reduced before it reaches a sufficiently reduced zone such as the low redox potentials that were measured in this study (Kirk 2005). This fact shows that the measurement of redox potential as an indicator of N species is inadequate. In the soils found at sites 1 and sites 2 reduction potentials almost never reached an aerobic state. No trends were seen between NO_3^- or NH_4^+ and redox potentials. This may be due to two factors; (1) soil NO_3^- is denitrified before it reaches a reduced zone and (2) redox potentials measured at the sites are too low for NH_4^+ mobilization and accumulation occurs. Micro-conditions occur in the rhizosphere along root systems to allow for the mobilization of NH_4^+ and the conversion of NO_3^-.

Due to the nitrogen cycle allowing the accumulation of NH_4^+ due to the lack of oxygen in wet soils, NH_4^+ may become a major source of N for plants through

nitrification in the rhizosphere. There is no conversion of NH_4^+ via the nitrogen cycle in bulk soil. Our results show that N concentrations in soil are not a limiting factor for plants.

5.5 Nitrogen, Species Diversity, and Elevation

No study has examined micro-elevation changes effect plant species diversity. In this study, the strong positive relationship between elevation and species richness is consistent with stressful conditions for roots in reduced soils. This is consistent with the suitable habitat conditions associated with oxidized soils (or less reducing) and the decrease in stress placed on root structures (Pezeshki and DeLaune 2012). As elevation increased, Eh values also increased. This study showed that small elevation changes significantly affected the chemical parameters that control species diversity.

A major difference between our study and Hopfensperger et al. (2009) is that could be the cause of the differences is the elevations changes that the nitrate was measured at. Hopfensperger et al. (2009) measured elevation changes of 70 cm at wetland sites spread across the 80 hectares of Dyke Marsh Preserve, South of Alexandria, Virginia (Hopfensperger et al. 2009). The elevations ranges in this study were 30 cm at very local distances from one another. While the elevations cannot be directly compared to one another, the ranges can. There would be significant differences in biogeochemistry and nutrient dynamics between an elevation ranging 30 cm while the other ranging 70 cm. A possible explanation for the differences in results are that nitrate is reduced before it even has time to reach or accumulate in the soil.

The Eh values across Sites 1 and 2 in this study were in a high reduction zones for majority of the time measured. Nitrate would be reduced to very low levels, resulting in a different finding than Hopfsenperger et al. 2009 A second explanation was that there was a greater species diversity in the higher elevations than in the lower elevations. The increase in species diversity in the higher elevations at Sites 1 and 2 could increase the demand for nitrates in the soil, thereby lowering the concentration found at the sampled locations

A negative trend was seen with species diversity and nitrate levels. As the species diversity increased, nitrate levels decreased. The correlation could be due to increase pressure on N supplies at that location. An increase in species diversity would increase the already high demand for soil nitrate (Kirk 2005; Mitsch and Gosselink 2007). This explains the trend that was seen between elevation and nitrate levels. As elevation increased, species diversity also increased. The increase in species diversity could be adding more competition for the already scarce nitrate in the soil thereby depleting it because of the large demand

Soil Eh values measured in this study were almost always in the denitrification or below denitrification zone. Due to the high levels of denitrification, NO_3^- will not be nitrified in the bulk soil. This suggests that all NO_3^- at sites 1 and 2 is due to nitrification in the rhizosphere, and any NO_3^- acquired by plants will take place in the rhizosphere. As stated before any NO_3^- added to the soil would be denitrified before it reaches any reduced zone.

Baldwin 2013 found in a field experiment that when plots were fertilized with NO_3^-, perennials abundance increased, while annual abundance decreased. The results in this report are not consistent with the findings of Baldwin 2013. But, the changes in NO_3^- across different elevations were not equal to the quantities of NO_3^- used to fertilize the plots in the Baldwin 2013 study. This could be why the small changes in NO_3^- levels would not affect ratios of perennials versus annuals. Large equilibrium shifts in nutrients such as nitrate may alter communities significantly. However, if concentrations remain low and unchanged systems would remain in balance.

Any N needed for plant growth was acquired via rhizosphere processes. Changes in these rhizosphere processes may be altered due to changes in sea level or changes in vegetation community structures, and could affected nutrient availability and ultimately N retention within tidal freshwater wetlands (Mitsch and Gosselink 2007, and Kirk 2005).

5.6 Vegetation Community Structure (Annuals versus Perennials)

Annual species could be the major factor contribution to overall wetland species diversity. Annual cover was high when perennial cover was low (Low elevation areas) and annual cover was low when perennial cover was high (high elevation areas) (r=-0.48) plots in this study were either dominated by annuals or by perennials.

These results may be controlled by abiotic factors. As elevation increased the annuals became less abundant. There was no trend that was seen between elevation and perennial organisms. This suggests that the annual appearance is most sensitive to

changes in elevation while perennials are not as influenced. This is supported by Hopfensperger et al. 2007 who showed perennial plants were found to be most affected by abiotic factors and allowed annuals to compete with them. Our results found that abiotic factors associated with elevation such as redox potential were lowest in low elevation areas. The stress associated with low redox potentials in low areas could be the factor that is allowing the high number of annuals to compete in the lower elevation areas. The mechanism for this may be increased habitat through the lower levels of perennials. Abiotic factors, such as low redox potentials, place stress on perennials that decrease their fitness and potentially create open patches. Annuals have been shown to colonize and rely on open patches (Mitsch and Gosselink 2007). Although evidence from Hopfensperger et al. 2007 supports this, the correlation between measured redox potentials and number of annuals or perennials was not significant.

There was no trend seen between annuals and nitrate levels or between perennials and nitrate levels. Annuals were shown to be negatively affected by increased nitrate levels (Baldwin 2013). However, nitrate levels in this study were very low so there was likely no mechanism for negatively affecting the completion between annuals and perennials for nitrogen.

5.6.1 Appearance of *A. virginica*.

Two individuals of *A. virginica* were found at site 2. No individuals were found within sampled locations, therefor correlations between the appearance *A. virginica* and physiochemical conditions could not be draw. Sampling was done in the fall to allow any

stands of *A. virginica* to appear. The sites in this study were chosen due to the appearance of *A. virginica* in previous years. The lack of appearance at the sites could be due to many environmental factors. These factors are not understood and should be considered in future studies of *A. virginica*.

6. Conclusion

The two main findings of this paper are (1) over micro elevation changes of 30 cm, biogeochemical conditions and nutrient availability are altered significantly and (2) changes to the physiochemical environment over the micro-elevations do alter vegetation community structures.

Within the Vandell Preserve, wetland elevation changes over 30 cm had significant changes in the chemical conditions that control wetland vegetation. The distribution of annuals and perennials were significantly affected by these changes suggesting that micro habitats do occur within the wetland. The appearance of annuals were affected by the small elevation changes, suggesting that annuals are sensitive to small changes in abiotic factors that are adding stress on perennials. Our data suggests that future alteration to wetland elevation would shift vegetation community structure and alter the balance of annuals and perennials. Develop each of these with specific predictions.

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